

METHODS FOR GLUCAGON SUPPRESSIONRELATED APPLICATIONS

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~~This application claims priority from U.S. Provisional~~
Application 60/116,380, entitled "Novel Exendin Agonist
5 Formulations And Methods Of Administration Thereof," filed
January 14, 1999 (and the corresponding PCT application
filed January 14, 2000, Serial No. [not yet assigned]), U.S.
Provisional Application 60/132,017, entitled "Methods for
Glucagon Suppression," filed April 30, 1999, and U.S.
10 Provisional Application 60/[not yet assigned], entitled "Use
of Exendins and Agonists Thereof for Modulation of
Triglyceride Levels and Treatment of Dyslipidemia," filed
January 10, 2000, the contents of which are hereby
~~incorporated by reference in their entirety.~~

FIELD OF THE INVENTION

15 The present invention relates to methods of suppressing
and/or lowering glucagon in a subject, comprising the
administration of an exendin, an exendin agonist, or a
20 modified exendin or exendin agonist having an exendin or
exendin agonist peptide linked to one or more polyethylene
glycol polymers or other compound useful to decrease renal
clearance of the parent peptide. Such methods are useful,
for example, in the treatment of hyperglucagonemia and other
25 conditions in which lower levels of glucagon or suppression
of glucagon secretion are of benefit.

BACKGROUND

30 The following description includes information that may
be useful in understanding the present invention. It is not
an admission that any of the information provided herein is
prior art to the presently claimed invention, or relevant,
nor that any of the publications specifically or implicitly
referenced are prior art.

The exendins are peptides that are found in the salivary secretions of the Gila monster and the Mexican Beaded Lizard, reptiles that are endogenous to Arizona and Northern Mexico. Exendin-3 [SEQ. ID. NO. 1: His Ser Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly Pro Ser Ser Gly Ala Pro Pro Pro Ser-NH₂] is present in the salivary secretions of *Heloderma horridum* (Mexican Beaded Lizard), and exendin-4 [SEQ. ID. NO. 2: His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly Pro Ser Ser Gly Ala Pro Pro Pro Ser-NH₂] is present in the salivary secretions of *Heloderma suspectum* (Gila monster) (Eng, J., et al., J. Biol. Chem., 265:20259-62, 1990; Eng, J., et al., J. Biol. Chem., 267:7402-05, 1992). The amino acid sequence of exendin-3 is shown in Figure 1. The amino acid sequence of exendin-4 is shown in Figure 2. Exendin-4 was first thought to be a (potentially toxic) component of the venom. It now appears that exendin-4 is devoid of toxicity, and that it instead is made in salivary glands in the Gila monster.

dis ~~The exendins have some sequence similarity to several members of the glucagon-like peptide family, with the highest homology, 53%, being to GLP-1[7-36]NH₂ [SEQ. ID. NO. 3] (Goke, et al., J. Biol. Chem., 268:19650-55, 1993). GLP-1[7-36]NH₂, also sometimes referred to as proglucagon[78-107] or simply "GLP-1" as used most often herein, has an insulinitropic effect, stimulating insulin secretion from pancreatic beta-cells; GLP-1 has also been reported to inhibit glucagon secretion from pancreatic alpha-cells (Ørskov, et al., Diabetes, 42:658-61, 1993; D'Alessio, et al., J. Clin. Invest., 97:133-38, 1996). GLP-1 has been reported to inhibit gastric emptying (Willms B, et al., J. Clin. Endocrinol Metab 81 (1): 327-32, 1996; Wettergren A, et~~

al., Dig Dis Sci 38 (4): 665-73, 1993), and gastric acid secretion (Schjoldager BT, et al., Dig Dis Sci 34 (5): 703-8, 1989; O'Halloran DJ, et al., J Endocrinol 126 (1): 169-73, 1990; Wettergren A, et al., Dig Dis Sci 38 (4): 665-73, 1993)). GLP-1[7-37], which has an additional glycine residue at its carboxy terminus, is reported to stimulate insulin secretion in humans (Ørskov, et al., Diabetes, 42:658-61, 1993). A transmembrane G-protein adenylate-cyclase-coupled receptor said to be responsible at least in part for the insulinotropic effect of GLP-1 has reportedly been cloned from a beta-cell line (Thorens, Proc. Natl. Acad. Sci. USA 89:8641-45, 1992). GLP-1 has been the focus of significant investigation in recent years due to its reported action on the amplification of stimulated insulin production (Byrne MM, Goke B. Lessons from human studies with glucagon-like peptide-1: Potential of the gut hormone for clinical use. In: Fehmann HC, Goke B. Insulinotropic Gut Hormone Glucagon-Like Peptide 1. Basel, Switzerland: ~~Harger, 1997:219-321~~).

Other reports relate to the inhibition of gastric emptying (Wettergren A, et al., Truncated GLP-1 (proglucagon 78-107-amide) inhibits gastric and pancreatic functions in man, Dig. Dis. Sci. 1993 Apr;38(4):665-73), inhibition of glucagon secretion (Creutzfeldt WOC, et al., Glucagonostatic actions and reduction of fasting hyperglycemia by exogenous glucagon-like peptide I(7-36) amide in type I diabetic patients, Diabetes Care 1996;19(6):580-6), and a purported role in appetite control (Turton MD, et al., A role for glucagon-like peptide-1 in the central regulation of feeding, Nature 1996 Jan;379(6560):69-72).

GLP-1 has also been reported to restore islet glucose sensitivity in aging rats, restoring their glucose tolerance to that of younger rats (Egan JM, et al., Glucagon-like

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peptide-1 restores acute-phase insulin release to aged rats, *Diabetologia* 1997 Jun;40(Suppl 1):A130). However, the short duration of biological action of GLP-1 *in vivo* is one feature of the peptide that has hampered its development as a therapeutic agent. Various methods have been tried to prolong the half-life of GLP-1 or GLP-1(7-37), including attempts to alter their amino acid sequence and to deliver them using certain formulations (see, e.g., European Patent Application, entitled "Prolonged Delivery of Peptides," by Darley, et al., publication number 0 619 322 A2, regarding the inclusion of polyethylene glycol in formulations containing GLP-1 (7-37)).

Pharmacological studies have led to reports that exendin-4 can act at GLP-1 receptors on certain insulin-secreting cells, at dispersed acinar cells from guinea pig pancreas, and at parietal cells from stomach; the peptide is also reported to stimulate somatostatin release and inhibit gastrin release in isolated stomachs (Goke, et al., J. Biol. Chem. 268:19650-55, 1993; Schepp, et al., Eur. J. Pharmacol., 69:183-91, 1994; Eissele, et al., Life Sci., 55:629-34, 1994). Exendin-3 and exendin-4 were reportedly found to stimulate cAMP production in, and amylase release from, pancreatic acinar cells (Malhotra, R., et al., Regulatory Peptides, 41:149-56, 1992; Raufman, et al., J. Biol. Chem. 267:21432-37, 1992; Singh, et al., Regul. Pept. 53:47-59, 1994). Additionally, exendin-4 has a significantly longer duration of action than GLP-1. For example, in one experiment, glucose lowering by exendin-4 in diabetic mice was reported to persist for several hours, and, depending on dose, for up to 24 hours (Eng J. Prolonged effect of exendin-4 on hyperglycemia of db/db mice, Diabetes 1996 May; 45(Suppl 2):152A (abstract 554)). Based on their insulinotropic activities, the use of exendin-3 and exendin-

4 for the treatment of diabetes mellitus and the prevention of hyperglycemia has been proposed (Eng, U.S. Patent No. 5,424,286).

5 The results of an investigation of whether exendins are the species homolog of mammalian GLP-1 was reported by Chen and Drucker who cloned the exendin gene from the Gila monster (J. Biol. Chem. 272(7):4108-15 (1997)). The observation that the Gila monster also has separate genes for proglucagons (from which GLP-1 is processed), that are 10 more similar to mammalian proglucagon than exendin, indicates that exendins are not merely species homologs of GLP-1.

To date, agents that serve to delay gastric emptying have generally found a place in medicine as diagnostic aids 15 in gastrointestinal radiological examinations. For example, glucagon is a polypeptide hormone that is produced by the alpha cells of the pancreatic islets of Langerhans. It is a hyperglycemic agent that mobilizes glucose by activating hepatic glycogenolysis. It can to a lesser extent stimulate 20 the secretion of pancreatic insulin. Glucagon is used in the treatment of insulin-induced hypoglycemia, for example, when administration of glucose intravenously is not possible. However, as glucagon reduces the motility of the gastro-intestinal tract it is also used as a diagnostic aid 25 in gastrointestinal radiological examinations. Glucagon has also been used in several studies to treat various painful gastrointestinal disorders associated with spasm. Daniel, et al. (Br. Med. J., 3:720, 1974) reported quicker symptomatic relief of acute diverticulitis in patients 30 treated with glucagon compared with those who had been treated with analgesics or antispasmodics. A review by Glauser, et al. (J. Am. Coll. Emergency Physns, 8:228, 1979) described relief of acute esophageal food obstruction

following glucagon therapy. In another study, glucagon significantly relieved pain and tenderness in 21 patients with biliary tract disease compared with 22 patients treated with placebo (M.J. Stower, et al., Br. J. Surg., 69:591-2, 1982).

Methods for regulating gastrointestinal motility using amylin agonists are described in commonly owned International Application No. PCT/US94/10225, published March 16, 1995.

Methods for regulating gastrointestinal motility using exendin agonists are described in commonly owned U.S. Patent Application Serial No. 08/908,867, filed August 8, 1997 entitled "Methods for Regulating Gastrointestinal Motility," which application is a continuation-in-part of U.S. Patent Application Serial No. 08/694,954, filed August 8, 1996.

Methods for reducing food intake using exendin agonists are described in commonly owned U.S. Patent Application Serial No. 09/003,869, filed January 7, 1998, entitled "Use of Exendin and Agonists Thereof for the Reduction of Food Intake," which claims the benefit of U.S. Provisional Application Nos. 60/034,905 filed January 7, 1997, 60/055,404 filed August 7, 1997, 60/065,442 filed November 14, 1997 and 60/066,029 filed November 14, 1997.

Novel exendin agonist compounds are described in commonly owned PCT Application Serial No. PCT/US98/16387 filed August 6, 1998, entitled "Novel Exendin Agonist Compounds," which claims the benefit of U.S. Patent Application Serial No. 60/055,404, filed August 8, 1997.

Other novel exendin agonists are described in commonly owned PCT Application Serial No. PCT/US98/24210, filed November 13, 1998, entitled "Novel Exendin Agonist Compounds," which claims the benefit of U.S. Provisional Application No. 60/065,442 filed November 14, 1997.

Still other novel exendin agonists are described in commonly owned PCT Application Serial No. PCT/US98/24273, filed November 13, 1998, entitled "Novel Exendin Agonist Compounds," which claims the benefit of U.S. Provisional Application No. 60/066,029 filed November 14, 1997.

Other recent advances in exendin related technology are described in U.S. Provisional Patent Application Serial No. 60/075,122, filed February 13, 1998, entitled "Inotropic and Diuretic Effects of Exendin and GLP-1" and in U.S. Provisional Patent Application Serial No. 60/116,380, filed January 14, 1998, entitled "Novel Exendin Agonist Formulations and Methods of Administration Thereof".

Polyethylene glycol (PEG) modification of therapeutic peptides and proteins may yield both advantages and disadvantages. While PEG modification may lead to improved circulation time, reduced antigenicity and immunogenicity, improved solubility, resistance to proteolysis, improved bioavailability, reduced toxicity, improved stability, and easier formulation of peptides (See, Francis et al., International Journal of Hematology, 68:1-18, 1998) problems with PEGylation in most cases is substantial reduction in bioactivity. Id. In addition, most methods involve use of linkers that have several types of adverse effects including immunogenicity, instability, toxicity, and reactivity. Id.

Glucagonoma (tumor of glucagon-secreting cells) produces, in addition to glucose intolerance, a skin condition, necrolytic migratory erythema. This is a raised scaly red rash, sometimes blistering and eventually crusting, localized to the face, abdomen, extremities and perineum. It can also be associated with inflammation of the tongue and mouth, and diseased nails and thinning of the hair. The condition is reported to respond to octreotide, a glucagonostatic hormone analog. The compounds described

herein are also useful as glucagonastatic agents and thus in the treatment of this disease, which was first described in 1966 (Kaplan, L.M. Endocrine Tumors of the Gastrointestinal Tract and Pancreas. Ch 262, p1392: In
5 Harrison's Principles of Internal Medicine, 12th Edition. McGraw-Hill Inc, New York, 1991). The compounds described herein that are useful for lowering glucagon levels and/or suppressing glucagon secretion include exendin, exendin agonists, and modified exendins and exendin agonists and
10 related formulations, and dosage formulations.

The contents of the above-identified articles, patents, and patent applications, and all other documents mentioned or cited herein, are hereby incorporated by reference in their entirety. Applicants reserve the right to physically
15 incorporate into this application any and all materials and information from any such articles, patents, patent applications, or other documents mentioned or cited herein.

SUMMARY OF THE INVENTION

20 The present invention relates to methods for lowering glucagon levels and/or suppressing glucagon secretion in a subject. It also relates to the treatment of hyperglucagonemia and conditions that benefit from administration of glucagonostatic agents, including but not
25 limited to necrolytic migratory erythema.

Thus, in one aspect, the invention relates to the use of an exendin, an exendin agonist, or a modified exendin or exendin agonist having an exendin or exendin agonist linked to one or more polyethylene glycol polymers, or other
30 molecular weight enhancing molecules, for lowering glucagon levels in a subject.

In another aspect, the invention relates to the use of an exendin, an exendin agonist, or a modified exendin or

exendin agonist having an exendin or exendin agonist linked to one or more polyethylene glycol polymers or other compounds useful to decrease renal clearance of the parent peptide, for suppressing glucagon secretion in a subject.

5 In still another aspect, the invention relates to the use of an exendin, an exendin agonist, or a modified exendin or exendin agonist having an exendin or exendin agonist linked to one or more polyethylene glycol polymers, or other molecular weight enhancing molecules, for treating
10 conditions associated with hyperglucagonemia.

In yet another aspect, the invention relates to the use of an exendin, an exendin agonist, or a modified exendin or exendin agonist having an exendin or exendin agonist linked to one or more polyethylene glycol polymers, or other
15 molecular weight enhancing molecules, for treating a subject with a glucagonoma or necrolytic migratory erythema.

In preferred embodiments, the exendin is exendin-4. In other preferred embodiments, the modified exendin or exendin agonist has a molecular weight that is greater than the
20 molecular weight of the exendin or exendin agonist (preferably about 10%, 50% or 90% greater), the modified exendin or exendin agonist has a negative charge that is greater than the negative charge of the exendin or exendin agonist (preferably about 10%, 50% or 90% greater), the
25 modified exendin or exendin agonist has a kidney clearance that is less than the kidney clearance of the exendin or exendin agonist (preferably about 10%, 50% or 90% less), the modified exendin or exendin agonist has a half-life that is greater than the half-life of the exendin or exendin agonist
30 (preferably about 10%, 50% or 90% greater), the modified exendin or exendin agonist has a immunogenicity/antigenicity that is less than the immunogenicity/antigenicity of the exendin or exendin agonist, the modified exendin or exendin

agonist has a solubility that is greater than the solubility of the exendin or exendin agonist (preferably about 10%, 50% or 90% greater), the modified exendin or exendin agonist has a proteolysis rate that is less than the proteolysis rate of the exendin or exendin agonist (preferably about 10%, 50% or 90% less), the modified exendin or exendin agonist has a toxicity that is less than the toxicity of the exendin or exendin agonist, the modified exendin or exendin agonist has a stability that is greater than the stability of the exendin or exendin agonist, and the modified exendin or exendin agonist has a permeability/biological function that is greater or less than the permeability/biological function of the exendin or exendin agonist (preferably about 10%, 50% or 90% greater or less).

The exendin or exendin agonist may be linked to one, two or three polyethylene glycol polymers. The polyethylene glycol polymers may preferably have molecular weights between 500 and 20,000. In a preferred embodiment, the modified exendin or exendin agonist is one of compounds 201-217, more preferably one of compounds 209, 210 and 213, or one of compounds 201 and 202, or one of compounds 216 and 217 (See Example 4 below).

The polyethylene glycol polymers are preferably linked to an amino, carboxyl, or thio group, and may be linked by N or C termini of side chains of lysine, aspartic acid, glutamic acid, or cysteine, or alternatively, the polyethylene glycol polymers may be linked with diamine and dicarboxylic groups. The exendin or exendin agonist is preferably linked to the polyethylene glycol polymers through an epsilon amino group on a lysine amino acid of the exendin or exendin agonist.

By "exendin agonist" is meant a compound which mimics the effects of exendins, e.g., on gastric motility and

gastric emptying (namely, a compound which effectively binds to the receptor at which exendins exert their action on gastric motility and gastric emptying, preferably an analog or derivative of an exendin) or a compound, e.g., that mimics the effects of exendin on the reduction of food intake by binding to the receptor or receptors where exendin causes this effect. Preferred exendin agonist compounds include those described in United States Patent Application Serial No. 90/003,869, entitled, "Use of Exendin And Agonists Thereof For The Reduction of Food Intake", filed January 7, 1998, (and the priority applications thereto) which enjoys common ownership with the present application and which is incorporated by this reference into the present application as though fully set forth herein. Effects of exendins or exendin agonists can be identified, evaluated, or screened for, using the methods described herein, or other methods known in the art for determining exendin effects.

In another aspect, a therapeutically effective amount of an amylin agonist is also administered to the subject. In a preferred aspect, the amylin agonist is an amylin or an amylin agonist analog such as ^{25,28,29}Pro-human-amylin. (also known as "pramlintide," and previously referred to as "AC-137" and described in "Amylin Agonist Peptides and Uses Therefor," U.S. Patent No. 5,686,511, issued November 11, 1997), or salmon calcitonin.

Preferably, the subject is a vertebrate, more preferably a mammal, and most preferably a human. In preferred aspects, the exendin, exendin agonist, or modified exendin or exendin agonist of the invention is administered parenterally, more preferably by injection. In a most preferred aspect, the injection is a peripheral injection. Preferably, about 1 µg-30 µg to about 5 mg of the modified exendin or exendin agonist of the invention is administered per day. More

preferably, about 1-30 μ g to about 2mg, or about 1-30 μ g to about 1mg of the modified exendin or exendin agonist of the invention is administered per day. Most preferably, about 3 μ g to about 500 μ g of the modified exendin or exendin agonist of the invention is administered per day.

Preferred exendins or exendin agonists for modification and use include:

exendin-4 (1-30) [SEQ ID NO 4: His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly];

exendin-4 (1-30) amide [SEQ ID NO 5: His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly-NH₂];

exendin-4 (1-28) amide [SEQ ID NO 6: His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn-NH₂];

¹⁴Leu, ²⁵Phe exendin-4 amide [SEQ ID NO 7: His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn Gly Gly Pro Ser Ser Gly Ala Pro Pro Pro Ser-NH₂];

¹⁴Leu, ²⁵Phe exendin-4 (1-28) amide [SEQ ID NO 8: His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂]; and

¹⁴Leu, ²²Ala, ²⁵Phe exendin-4 (1-28) amide [SEQ ID NO 9: His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu Glu Ala Val Arg Leu Ala Ile Glu Phe Leu Lys Asn-NH₂].

Definitions

In accordance with the present invention and as used herein, the following terms are defined to have the following meanings, unless explicitly stated otherwise.

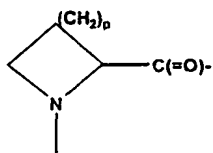
The term "amino acid" refers to natural amino acids, unnatural amino acids, and amino acid analogs, all in their

D and L stereoisomers if their structure allow such stereoisomeric forms. Natural amino acids include alanine (Ala), arginine (Arg), asparagine (Asn), aspartic acid (Asp), cysteine (Cys), glutamine (Gln), glutamic acid (Glu),
5 glycine (Gly), histidine (His), isoleucine (Ile), leucine (Leu), Lysine (Lys), methionine (Met), phenylalanine (Phe), proline (Pro), serine (Ser), threonine (Thr), typtophan (Trp), tyrosine (Tyr) and valine (Val). Unnatural amino acids include, but are not limited to azetidinecarboxylic
10 acid, 2-aminoadipic acid, 3-aminoadipic acid, beta-alanine, aminopropionic acid, 2-aminobutyric acid, 4-aminobutyric acid, 6-aminocaproic acid, 2-aminoheptanoic acid, 2-aminoisobutyric acid, 3-aminoisbutyric acid, 2-aminopimelic acid, tertiary-butylglycine, 2,4-diaminoisobutyric acid,
15 desmosine, 2,2'-diaminopimelic acid, 2,3-diaminopropionic acid, N-ethylglycine, N-ethylasparagine, homoproline, hydroxylysine, allo-hydroxylysine, 3-hydroxyproline, 4-hydroxyproline, isodesmosine, allo-isoleucine, N-methylalanine, N-methylglycine, N-methylisoleucine, N-
20 methylpentylglycine, N-methylvaline, naphthalanine, norvaline, norleucine, ornithine, pentylglycine, pipecolic acid and thioproline. Amino acid analogs include the natural and unnatural amino acids which are chemically blocked, reversibly or irreversibly, or modified on their N-
25 terminal amino group or their side-chain groups, as for example, methionine sulfoxide, methionine sulfone, S-(carboxymethyl)-cysteine, S-(carboxymethyl)-cysteine sulfoxide and S-(carboxymethyl)-cysteine sulfone.

The term "amino acid analog" refers to an amino acid
30 wherein either the C-terminal carboxy group, the N-terminal amino group or side-chain functional group has been chemically codified to another functional group. For example, aspartic acid-(beta-methyl ester) is an amino acid

analog of aspartic acid; N-ethylglycine is an amino acid analog of glycine; or alanine carboxamide is an amino acid analog of alanine.

The term "amino acid residue" refers to radicals having the structure: (1) $-C(O)-R-NH-$, wherein R typically is $-CH(R')-$, wherein R' is an amino acid side chain, typically H or a carbon containing substituent;



or (2) , wherein p is 1, 2 or 3 representing the azetidinecarboxylic acid, proline or pipecolic acid residues, respectively.

The term "lower" referred to herein in connection with organic radicals such as alkyl groups defines such groups with up to and including about 6, preferably up to and including 4 and advantageously one or two carbon atoms. Such groups may be straight chain or branched chain.

"Pharmaceutically acceptable salt" includes salts of the compounds of the present invention derived from the combination of such compounds and an organic or inorganic acid. In practice the use of the salt form amounts to use of the base form. The compounds of the present invention are useful in both free base and salt form, with both forms being considered as being within the scope of the present invention.

In addition, the following abbreviations stand for the following:

"ACN" or "CH₃CN" refers to acetonitrile.

"Boc", "tBoc" or "Tboc" refers to t-butoxy carbonyl.

"DCC" refers to N,N'-dicyclohexylcarbodiimide.

"Fmoc" refers to fluorenylmethoxycarbonyl.

"HBTU" refers to 2-(1H-benzotriazol-1-yl)-

1,1,3,3,-tetramethyluronium hexafluorophosphate.

"HOBt" refers to 1-hydroxybenzotriazole monohydrate.

"homoP" or hPro" refers to homoproline.

"MeAla" or "Nme" refers to N-methylalanine.

5 "naph" refers to naphthylalanine.

"pG" or pGly" refers to pentylglycine.

"tBuG" refers to tertiary-butylglycine.

"ThioP" or tPro" refers to thioproline.

"3Hyp" refers to 3-hydroxyproline

10 "4Hyp" refers to 4-hydroxyproline

"NAG" refers to N-alkylglycine

"NAPG" refers to N-alkylpentylglycine

"Norval" refers to norvaline

"Norleu" refers to norleucine

15 Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

20 Figure 1 depicts the amino acid sequence for exendin-3 [SEQ. ID. NO. 1].

Figure 2 depicts the amino acid sequence for exendin-4 [SEQ. ID. NO. 2].

Chs B21
25 ~~Figure 3 depicts the amino acid sequences for certain exendin agonist compounds useful in the present invention [SEQ. ID. NOS. 10 TO 40].~~

Chs B31
~~Figure 4 depicts the amino acid sequences for certain compounds of the present invention, Compounds 1-174.~~

30 Figure 5 is a graph showing the effect of functional nephrectomy on exendin-4 clearance.

Figure 6 is a graph showing the terminal decay of exendin-4 plasma levels in nephrectomized and sham subjects.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to relates to methods of suppressing and/or lowering glucagon in a subject, comprising the administration of an exendin, an exendin
5 agonist, or a modified exendin or exendin agonist having an exendin or exendin agonist peptide linked to one or more polyethylene glycol polymers or other compound useful to increase molecular weight. Such methods are useful, for example, in the treatment of hyperglucagonemia and other
10 conditions in which lower levels of glucagon or suppression of glucagon secretion are of benefit. Such conditions include, but are not limited to, glucagonoma and necrolytic migratory erythema.

15 Modified Exendins And Exendin Agonists

The modified exendins and exendin agonists of the present invention include, for example, one or more PEG polymers linked to an exendin or exendin agonist, such as a naturally occurring exendin, a synthetic exendin or an
20 exendin agonist.

Exendin-4

Exendin-4 is a naturally occurring peptide isolated from the salivary secretions of the Gila monster. Animal
25 testing of exendin-4 has shown that its ability to lower blood glucose persists for several hours. Exendin-4, a 39-amino acid polypeptide, is synthesized using solid phase synthesis as described herein, and this synthetic material has been shown to be identical to that of native exendin-4.

30 As described herein, the nonclinical pharmacology of exendin-4 has been studied. In the brain, exendin-4 binds principally to the area postrema and nucleus tractus solitarius region in the hindbrain and to the subfornical

organ in the forebrain. Exendin-4 binding has been observed in the rat and mouse brain and kidney. The structures to which exendin-4 binds in the kidney are unknown.

Various experiments have compared the biologic actions of exendin-4 and GLP-1 and demonstrated a more favorable spectrum of properties for exendin-4. A single subcutaneous dose of exendin-4 lowered plasma glucose in *db/db* (diabetic) and *ob/ob* (diabetic obese) mice by up to 40%. In Diabetic Fatty Zucker (ZDF) rats, 5 weeks of treatment with exendin-4 lowered HbA_{1c} (a measure of glycosylated hemoglobin used to evaluate plasma glucose levels) by up to 41%. Insulin sensitivity was also improved by 76% following 5 weeks of treatment in obese ZDF rats. In glucose intolerant primates, dose-dependent decreases in plasma glucose were also observed.

An insulintropic action of exendin-4 has also been observed in rodents, improving insulin response to glucose by over 100% in non-fasted Harlan Sprague Dawley (HSD) rats, and by up to ~10-fold in non-fasted *db/db* mice. Higher pretreatment plasma glucose concentrations were associated with greater glucose-lowering effects. Thus the observed glucose lowering effect of exendin-4 appears to be glucose-dependent, and minimal if animals are already euglycemic.

Exendin-4 dose dependently slowed gastric emptying in HSD rats and was ~90-fold more potent than GLP-1 for this action. Exendin-4 has also been shown to reduce food intake in NIH/Sw (Swiss) mice following peripheral administration, and was at least 1000 times more potent than GLP-1 for this action. Exendin-4 reduced plasma glucagon concentrations by approximately 40% in anesthetized ZDF rats during hyperinsulinemic, hyperglycemic clamp conditions, but did not affect plasma glucagon concentrations during euglycemic conditions in normal rats. Exendin-4 has been shown to

dose-dependently reduce body weight in obese ZDF rats, while in lean ZDF rats, the observed decrease in body weight appears to be transient.

Through effects on lowering glucagon and suppressing glucagon secretion, exendins, exendin agonists, and modified exendins or exendin agonists containing exendin-4, for example, will be useful in people who would benefit from lowered glucagon, for example, people with glucagonoma and necrolytic migratory erythema, and people with diabetes whether or not they retain the ability to secrete insulin. See Example 5.

The toxicology of exendin-4 has been investigated in single-dose studies in mice, rats and monkeys, repeated-dose (up to 28 consecutive daily doses) studies in rats and monkeys and *in vitro* tests for mutagenicity and chromosomal alterations. To date, no deaths have occurred, and there have been no observed treatment-related changes in hematology, clinical chemistry, or gross or microscopic tissue changes. Exendin-4 was demonstrated to be non-mutagenic, and did not cause chromosomal aberrations at the concentrations tested (up to 5000 µg/mL).

ds BYL ~~In support of the investigation of the nonclinical pharmacokinetics and metabolism of exendin-4, a number of immunoassays have been developed. A radioimmunoassay with limited sensitivity (~100 pM) was used in initial pharmacokinetic studies. A two-site IRMA assay for exendin-4 was subsequently validated with a lower limit of quantitation of 15 pM. The bioavailability of exendin-4, given subcutaneously, was found to be approximately 50-80% using the radioimmunoassay. This was similar to that seen following intraperitoneal administration (48-60%). Peak plasma concentrations (C_{max}) occurred between 30 and 43 minutes (T_{max}). Both C_{max} and AUC values were monotonically~~

~~related to dose. The apparent terminal half-life for~~
exendin-4 given subcutaneously was approximately 90-110
minutes. This was significantly longer than the 14-41
minutes seen following intravenous dosing. Similar results
5 were obtained using the IRMA assay. Degradation studies
with exendin-4 compared to GLP-1 indicate that exendin-4 is
~~relatively resistant to degradation.~~

Exendin Agonists

10 The structure activity relationship (SAR) of exendin
was investigated for structures that may relate to the
antidiabetic activity of exendin, for its stability to
metabolism, and for improvement of its physical
characteristics, especially as it pertains to peptide
15 stability and to amenability to alternative delivery
systems, and various exendin agonist peptide compounds have
been invented. Exendin agonists include exendin peptide
analogues in which one or more naturally occurring amino acids
are eliminated or replaced with another amino acid(s).
20 Preferred exendin agonists are agonist analogues of exendin-4.
Particularly preferred exendin agonists include those
described in commonly owned PCT Application Serial No.
PCT/US98/16387 filed August 6, 1998, entitled "Novel Exendin
Agonist Compounds," which claims the benefit of U.S. Patent
25 Application Serial No. 60/055,404, filed August 8, 1997;
commonly owned PCT Application Serial No. PCT/US98/24210,
filed November 13, 1998, entitled "Novel Exendin Agonist
Compounds," which claims the benefit of U.S. Provisional
Application No. 60/065,442 filed November 14, 1997; and,
30 commonly owned PCT Application Serial No. PCT/US98/24273,
filed November 13, 1998, entitled "Novel Exendin Agonist
Compounds," which claims the benefit of U.S. Provisional
Application No. 60/066,029 filed November 14, 1997, all of

which are incorporated herein by reference in their entirety, including any drawings.

Activity as exendin agonists can be indicated, for example, by activity in the assays described below. Effects of exendins or exendin agonists on gastric motility and gastric emptying can be identified, evaluated, or screened for, using the methods described herein, or other art-known or equivalent methods for determining gastric motility. Negative receptor assays or screens for exendin agonist compounds or candidate exendin agonist compounds, such as an amylin receptor assay/screen using an amylin receptor preparation as described in U.S. Patent No. 5,264,372, issued November 23, 1993, the contents of which are incorporated herein by reference, one or more calcitonin receptor assays/screens using, for example, T47D and MCF7 breast carcinoma cells, which contain calcium receptors coupled to the stimulation of adenylyl cyclase activity, and/or a CGRP receptor assay/screen using, for example, SK-N-MC cells.

One such method for use in identifying or evaluating the ability of a compound to slow gastric motility, involves: (a) bringing together a test sample and a test system, the test sample containing one or more test compounds, the test system containing a system for evaluating gastric motility, the system being characterized in that it exhibits, for example, elevated plasma glucose in response to the introduction to the system of glucose or a meal; and, (b) determining the presence or amount of a rise in plasma glucose in the system. Positive and/or negative controls may be used as well.

Also included within the scope of the present invention are pharmaceutically acceptable salts of the compounds of

formula (I-VIII) and pharmaceutical compositions including said compounds and salts thereof.

FORMULA I

- 5 Exendin agonist compounds also include those described in U.S. Provisional Application No. 60/065,442, including compounds of the formula (I) [SEQ ID NO. 41]:
- Xaa₁ Xaa₂ Xaa₃ Gly Xaa₅ Xaa₆ Xaa₇ Xaa₈ Xaa₉ Xaa₁₀
Xaa₁₁ Xaa₁₂ Xaa₁₃ Xaa₁₄ Xaa₁₅ Xaa₁₆ Xaa₁₇ Ala Xaa₁₉ Xaa₂₀
10 Xaa₂₁ Xaa₂₂ Xaa₂₃ Xaa₂₄ Xaa₂₅ Xaa₂₆ Xaa₂₇ Xaa₂₈-Z₁; wherein
- Xaa₁ is His, Arg or Tyr;
Xaa₂ is Ser, Gly, Ala or Thr;
Xaa₃ is Asp or Glu;
15 Xaa₅ is Ala or Thr;
Xaa₆ is Ala, Phe, Tyr or naphthylalanine;
Xaa₇ is Thr or Ser;
Xaa₈ is Ala, Ser or Thr;
Xaa₉ is Asp or Glu;
20 Xaa₁₀ is Ala, Leu, Ile, Val, pentylglycine or Met;
Xaa₁₁ is Ala or Ser;
Xaa₁₂ is Ala or Lys;
Xaa₁₃ is Ala or Gln;
Xaa₁₄ is Ala, Leu, Ile, pentylglycine, Val or Met;
25 Xaa₁₅ is Ala or Glu;
Xaa₁₆ is Ala or Glu;
Xaa₁₇ is Ala or Glu;
Xaa₁₉ is Ala or Val;
Xaa₂₀ is Ala or Arg;
30 Xaa₂₁ is Ala or Leu;
Xaa₂₂ is Ala, Phe, Tyr or naphthylalanine;
Xaa₂₃ is Ile, Val, Leu, pentylglycine, tert-butylglycine
or Met;

- Xaa₂₄ is Ala, Glu or Asp;
Xaa₂₅ is Ala, Trp, Phe, Tyr or naphthylalanine;
Xaa₂₆ is Ala or Leu;
Xaa₂₇ is Ala or Lys;
5 Xaa₂₈ is Ala or Asn;
Z₁ is -OH,
-NH₂
Gly-Z₂,
Gly Gly-Z₂,
10 Gly Gly Xaa₃₁-Z₂,
Gly Gly Xaa₃₁ Ser-Z₂,
Gly Gly Xaa₃₁ Ser Ser-Z₂,
Gly Gly Xaa₃₁ Ser Ser Gly-Z₂,
Gly Gly Xaa₃₁ Ser Ser Gly Ala-Z₂,
15 Gly Gly Xaa₃₁ Ser Ser Gly Ala Xaa₃₆-Z₂,
Gly Gly Xaa₃₁ Ser Ser Gly Ala Xaa₃₆ Xaa₃₇-Z₂ or
Gly Gly Xaa₃₁ Ser Ser Gly Ala Xaa₃₆ Xaa₃₇ Xaa₃₈-Z₂;
Xaa₃₁, Xaa₃₆, Xaa₃₇ and Xaa₃₈ are independently Pro,
homoproline, 3Hyp, 4Hyp, thioproline,
20 N-alkylglycine, N-alkylpentylglycine or
N-alkylalanine; and
Z₂ is -OH or -NH₂;
provided that no more than three of Xaa₃, Xaa₅, Xaa₆, Xaa₈,
Xaa₁₀, Xaa₁₁, Xaa₁₂, Xaa₁₃, Xaa₁₄, Xaa₁₅, Xaa₁₆, Xaa₁₇, Xaa₁₉,
25 Xaa₂₀, Xaa₂₁, Xaa₂₄, Xaa₂₅, Xaa₂₆, Xaa₂₇ and Xaa₂₈ are Ala.
Preferred N-alkyl groups for N-alkylglycine, N-
alkylpentylglycine and N-alkylalanine include lower alkyl
groups preferably of 1 to about 6 carbon atoms, more
preferably of 1 to 4 carbon atoms.
30 Preferred extendin agonist compounds include those
wherein Xaa₁ is His or Tyr. More preferably Xaa₁ is His.
Preferred are those compounds wherein Xaa₂ is Gly.

Preferred are those compounds wherein Xaa₁₄ is Leu, pentylglycine or Met.

Preferred compounds are those wherein Xaa₂₅ is Trp or Phe.

- 5 Preferred compounds are those where Xaa₆ is Phe or naphthylalanine; Xaa₂₂ is Phe or naphthylalanine and Xaa₂₃ is Ile or Val.

- Preferred are compounds wherein Xaa₃₁, Xaa₃₆, Xaa₃₇ and Xaa₃₈ are independently selected from Pro, homoproline, thioproline and N-alkylalanine.
- 10

Preferably Z₁ is -NH₂.

Preferably Z₂ is -NH₂.

- According to one aspect, preferred are compounds of formula (I) wherein Xaa₁ is His or Tyr, more preferably His; Xaa₂ is Gly; Xaa₆ is Phe or naphthylalanine; Xaa₁₄ is Leu, pentylglycine or Met; Xaa₂₂ is Phe or naphthylalanine; Xaa₂₃ is Ile or Val; Xaa₃₁, Xaa₃₆, Xaa₃₇ and Xaa₃₈ are independently selected from Pro, homoproline, thioproline or N-alkylalanine. More preferably Z₁ is -NH₂.
- 15

- 20 According to an especially preferred aspect, especially preferred compounds include those of formula (I) wherein: Xaa₁ is His or Arg; Xaa₂ is Gly or Ala; Xaa₃ is Asp or Glu; Xaa₅ is Ala or Thr; Xaa₆ is Ala, Phe or naphthylalanine; Xaa₇ is Thr or Ser; Xaa₈ is Ala, Ser or Thr; Xaa₉ is Asp or Glu; Xaa₁₀ is Ala, Leu or pentylglycine; Xaa₁₁ is Ala or Ser; Xaa₁₂ is Ala or Lys; Xaa₁₃ is Ala or Gln; Xaa₁₄ is Ala, Leu or pentylglycine; Xaa₁₅ is Ala or Glu; Xaa₁₆ is Ala or Glu; Xaa₁₇ is Ala or Glu; Xaa₁₉ is Ala or Val; Xaa₂₀ is Ala or Arg; Xaa₂₁ is Ala or Leu; Xaa₂₂ is Phe or naphthylalanine; Xaa₂₃ is Ile, Val or tert-butylglycine; Xaa₂₄ is Ala, Glu or Asp; Xaa₂₅ is Ala, Trp or Phe; Xaa₂₆ is Ala or Leu; Xaa₂₇ is Ala or Lys; Xaa₂₈ is Ala or Asn; Z₁ is -OH, -NH₂, Gly-Z₂, Gly Gly-Z₂, Gly Gly Xaa₃₁-Z₂, Gly Gly Xaa₃₁ Ser-Z₂, Gly Gly Xaa₃₁ Ser Ser-Z₂,
- 25
- 30

Gly Gly Xaa₃₁ Ser Ser Gly-Z₂, Gly Gly Xaa₃₁ Ser Ser Gly Ala-Z₂, Gly Gly Xaa₃₁ Ser Ser Gly Ala Xaa₃₆-Z₂, Gly Gly Xaa₃₁ Ser Ser Gly Ala Xaa₃₆ Xaa₃₇-Z₂, Gly Gly Xaa₃₁ Ser Ser Gly Ala Xaa₃₆ Xaa₃₇ Xaa₃₈-Z₂; Xaa₃₁, Xaa₃₆, Xaa₃₇ and Xaa₃₈ being independently
5 Pro homoproline, thioproline or N-methylalanine; and Z₂ being -OH or -NH₂; provided that no more than three of Xaa₃, Xaa₅, Xaa₆, Xaa₈, Xaa₁₀, Xaa₁₁, Xaa₁₂, Xaa₁₃, Xaa₁₄, Xaa₁₅, Xaa₁₆, Xaa₁₇, Xaa₁₉, Xaa₂₀, Xaa₂₁, Xaa₂₄, Xaa₂₅, Xaa₂₆, Xaa₂₇ and Xaa₂₈ are Ala. Especially preferred compounds include those set
10 forth in PCT application Serial No. PCT/US98/24210, filed November 13, 1998, entitled "Novel Exendin Agonist Compounds" identified therein as compounds 2-23.

According to an especially preferred aspect, provided are compounds where Xaa₁₄ is Leu, Ile, Val or pentylglycine,
15 more preferably Leu or pentylglycine, and Xaa₂₅ is Phe, Tyr or naphthylalanine, more preferably Phe or naphthylalanine. These compounds will be less susceptible to oxidative degradation, both in vitro and in vivo, as well as during synthesis of the compound.

20

FORMULA II

Exendin agonist compounds also include those described in U.S. Provisional Application No. 60/066,029, including compounds of the formula (II) [SEQ ID NO. 42]:

25 Xaa₁ Xaa₂ Xaa₃ Xaa₄ Xaa₅ Xaa₆ Xaa₇ Xaa₈ Xaa₉ Xaa₁₀
Xaa₁₁ Xaa₁₂ Xaa₁₃ Xaa₁₄ Xaa₁₅ Xaa₁₆ Xaa₁₇ Ala Xaa₁₉ Xaa₂₀
Xaa₂₁ Xaa₂₂ Xaa₂₃ Xaa₂₄ Xaa₂₅ Xaa₂₆ Xaa₂₇ Xaa₂₈-Z₁; wherein

Xaa₁ is His, Arg, Tyr, Ala, Norval, Val
30 or Norleu;

Xaa₂ is Ser, Gly, Ala or Thr;

Xaa₃ is Ala, Asp or Glu;

Xaa₄ is Ala, Norval, Val, Norleu or Gly;

- Xaa₅ is Ala or Thr;
Xaa₆ is Phe, Tyr or naphthylalanine;
Xaa₇ is Thr or Ser;
Xaa₈ is Ala, Ser or Thr;
5 Xaa₉ is Ala, Norval, Val, Norleu, Asp or Glu;
Xaa₁₀ is Ala, Leu, Ile, Val, pentylglycine or Met;
Xaa₁₁ is Ala or Ser;
Xaa₁₂ is Ala or Lys;
Xaa₁₃ is Ala or Gln;
10 Xaa₁₄ is Ala, Leu, Ile, pentylglycine, Val or Met;
Xaa₁₅ is Ala or Glu;
Xaa₁₆ is Ala or Glu;
Xaa₁₇ is Ala or Glu;
Xaa₁₉ is Ala or Val;
15 Xaa₂₀ is Ala or Arg;
Xaa₂₁ is Ala or Leu;
Xaa₂₂ is Phe, Tyr or naphthylalanine;
Xaa₂₃ is Ile, Val, Leu, pentylglycine, tert-butylglycine or
Met;
20 Xaa₂₄ is Ala, Glu or Asp;
Xaa₂₅ is Ala, Trp, Phe, Tyr or naphthylalanine;
Xaa₂₆ is Ala or Leu;
Xaa₂₇ is Ala or Lys;
Xaa₂₈ is Ala or Asn;
25 Z₁ is -OH,
-NH₂,
Gly-Z₂,
Gly Gly-Z₂,
Gly Gly Xaa₃₁-Z₂,
30 Gly Gly Xaa₃₁ Ser-Z₂,
Gly Gly Xaa₃₁ Ser Ser-Z₂,
Gly Gly Xaa₃₁ Ser Ser Gly-Z₂,
Gly Gly Xaa₃₁ Ser Ser Gly Ala-Z₂,

Gly Gly Xaa₃₁ Ser Ser Gly Ala Xaa₃₆-Z₂,
Gly Gly Xaa₃₁ Ser Ser Gly Ala Xaa₃₆ Xaa₃₇-Z₂,
Gly Gly Xaa₃₁ Ser Ser Gly Ala Xaa₃₆ Xaa₃₇ Xaa₃₈-Z₂ or
Gly Gly Xaa₃₁ Ser Ser Gly Ala Xaa₃₆ Xaa₃₇ Xaa₃₈ Xaa₃₉-Z₂;
5 wherein

Xaa₃₁, Xaa₃₆, Xaa₃₇ and Xaa₃₈ are independently
Pro, homoproline, 3Hyp, 4Hyp, thioproline,
N-alkylglycine, N-alkylpentylglycine or
N-alkylalanine; and

10 Z₂ is -OH or -NH₂;

provided that no more than three of Xaa₃, Xaa₄, Xaa₅, Xaa₆,
Xaa₈, Xaa₉, Xaa₁₀, Xaa₁₁, Xaa₁₂, Xaa₁₃, Xaa₁₄, Xaa₁₅, Xaa₁₆,
Xaa₁₇, Xaa₁₉, Xaa₂₀, Xaa₂₁, Xaa₂₄, Xaa₂₅, Xaa₂₆, Xaa₂₇ and Xaa₂₈
are Ala; and provided also that, if Xaa₁ is His, Arg or Tyr,
15 then at least one of Xaa₃, Xaa₄ and Xaa₉ is Ala.

Preferred N-alkyl groups for N-alkylglycine, N-
alkylpentylglycine and N-alkylalanine include lower alkyl
groups preferably of 1 to about 6 carbon atoms, more
preferably of 1 to 4 carbon atoms. Suitable compounds of
20 formula (II) include those described in application Serial
No. PCT/US98/24273, filed November 13, 1998, entitled "Novel
Exendin Agonist Compounds", identified therein in Examples
1-89 ("Compounds 1-89," respectively), as well as those
corresponding compounds identified therein in Examples 104
25 and 105.

Preferred such exendin agonist compounds include those
wherein Xaa₁ is His, Ala or Norval. More preferably Xaa₁ is
His or Ala. Most preferably Xaa₁ is His.

Preferred are those compounds of formula (II) wherein
30 Xaa₂ is Gly.

Preferred are those compounds of formula (II) wherein
Xaa₃ is Ala.

Preferred are those compounds of formula (II) wherein Xaa₄ is Ala.

Preferred are those compounds of formula (II) wherein Xaa₉ is Ala.

- 5 Preferred are those compounds of formula (II) wherein Xaa₁₄ is Leu, pentylglycine or Met.

Preferred compounds of formula (II) are those wherein Xaa₂₅ is Trp or Phe.

- 10 Preferred compounds of formula (II) are those where Xaa₆ is Ala, Phe or naphthylalanine; Xaa₂₂ is Phe or naphthylalanine; and Xaa₂₃ is Ile or Val.

Preferred are compounds of formula (II) wherein Xaa₃₁, Xaa₃₆, Xaa₃₇ and Xaa₃₈ are independently selected from Pro, homoproline, thioproline and N-alkylalanine.

- 15 Preferably Z₁ is -NH₂.

Preferably Z₂ is -NH₂.

- According to one aspect, preferred are compounds of formula (II) wherein Xaa₁ is Ala, His or Tyr, more preferably Ala or His; Xaa₂ is Ala or Gly; Xaa₆ is Phe or naphthylalanine; Xaa₁₄ is Ala, Leu, pentylglycine or Met; Xaa₂₂ is Phe or naphthylalanine; Xaa₂₃ is Ile or Val; Xaa₃₁, Xaa₃₆, Xaa₃₇ and Xaa₃₈ are independently selected from Pro, homoproline, thioproline or N-alkylalanine; and Xaa₃₉ is Ser or Tyr, more preferably Ser. More preferably Z₁ is -NH₂.

- 25 According to an especially preferred aspect, especially preferred compounds include those of formula (II) wherein: Xaa₁ is His or Ala; Xaa₂ is Gly or Ala; Xaa₃ is Ala, Asp or Glu; Xaa₄ is Ala or Gly; Xaa₅ is Ala or Thr; Xaa₆ is Phe or naphthylalanine; Xaa₇ is Thr or Ser; Xaa₈ is Ala, Ser or Thr; Xaa₉ is Ala, Asp or Glu; Xaa₁₀ is Ala, Leu or pentylglycine; Xaa₁₁ is Ala or Ser; Xaa₁₂ is Ala or Lys; Xaa₁₃ is Ala or Gln; Xaa₁₄ is Ala, Leu, Met or pentylglycine; Xaa₁₅ is Ala or Glu; Xaa₁₆ is Ala or Glu; Xaa₁₇ is Ala or Glu; Xaa₁₉ is Ala or Val;
- 30

Xaa₂₀ is Ala or Arg; Xaa₂₁ is Ala or Leu; Xaa₂₂ is Phe or naphthylalanine; Xaa₂₃ is Ile, Val or tert-butylglycine; Xaa₂₄ is Ala, Glu or Asp; Xaa₂₅ is Ala, Trp or Phe; Xaa₂₆ is Ala or Leu; Xaa₂₇ is Ala or Lys; Xaa₂₈ is Ala or Asn; Z₁ is -OH, -NH₂, Gly-Z₂, Gly Gly-Z₂, Gly Gly Xaa₃₁-Z₂, Gly Gly Xaa₃₁ Ser-Z₂, Gly Gly Xaa₃₁ Ser Ser-Z₂, Gly Gly Xaa₃₁ Ser Ser Gly-Z₂, Gly Gly Xaa₃₁ Ser Ser Gly Ala-Z₂, Gly Gly Xaa₃₁ Ser Ser Gly Ala Xaa₃₆-Z₂, Gly Gly Xaa₃₁ Ser Ser Gly Ala Xaa₃₆ Xaa₃₇-Z₂, Gly Gly Xaa₃₁ Ser Ser Gly Ala Xaa₃₆ Xaa₃₇ Xaa₃₈-Z₂ or Gly Gly Xaa₃₁ Ser Ser Gly Ala Xaa₃₆ Xaa₃₇ Xaa₃₈ Xaa₃₉-Z₂; Xaa₃₁, Xaa₃₆, Xaa₃₇ and Xaa₃₈ being independently Pro homoproline, thioproline or N-methylalanine; and Z₂ being -OH or -NH₂; provided that no more than three of Xaa₃, Xaa₅, Xaa₆, Xaa₈, Xaa₁₀, Xaa₁₁, Xaa₁₂, Xaa₁₃, Xaa₁₄, Xaa₁₅, Xaa₁₆, Xaa₁₇, Xaa₁₉, Xaa₂₀, Xaa₂₁, Xaa₂₄, Xaa₂₅, Xaa₂₆, Xaa₂₇ and Xaa₂₈ are Ala; and provided also that, if Xaa₁ is His, Arg or Tyr, then at least one of Xaa₃, Xaa₄ and Xaa₉ is Ala. Especially preferred compounds of formula (II) include those described in application Serial No. PCT/US98/24273, filed November 13, 1998, entitled "Novel Exendin Agonist Compounds" as having the amino acid sequence of SEQ. ID. NOS. 5-93 therein.

According to an especially preferred aspect, provided are compounds of formula (II) where Xaa₁₄ is Ala, Leu, Ile, Val or pentylglycine, more preferably Leu or pentylglycine, and Xaa₂₅ is Ala, Phe, Tyr or naphthylalanine, more preferably Phe or naphthylalanine. These compounds will be less susceptible to oxidative degradation, both in vitro and in vivo, as well as during synthesis of the compound.

30 FORMULA III

Also within the scope of the present invention are narrower genera of compounds having peptides of various lengths, for example genera of compounds which do not

include peptides having a length of 28, 29 or 30 amino acid residues, respectively. Additionally, the present invention includes narrower genera of compounds described in PCT application Serial No. PCT/US98/24210, filed November 13, 1998, entitled "Novel Exendin Agonist Compounds" and having particular amino acid sequences, for example, compounds of the formula (III) [SEQ. ID. NO. 43]:

Xaa₁ Xaa₂ Xaa₃ Gly Xaa₅ Xaa₆ Xaa₇ Xaa₈ Xaa₉ Xaa₁₀
10 Xaa₁₁ Xaa₁₂ Xaa₁₃ Xaa₁₄ Xaa₁₅ Xaa₁₆ Xaa₁₇ Ala Xaa₁₈ Xaa₁₉
Xaa₂₀ Xaa₂₁ Xaa₂₂ Xaa₂₃ Xaa₂₄ Xaa₂₅ Xaa₂₆ Xaa₂₇ Xaa₂₈-Z₁;

wherein

- Xaa₁ is His or Arg;
15 Xaa₂ is Gly or Ala;
Xaa₃ is Asp or Glu;
Xaa₅ is Ala or Thr;
Xaa₆ is Ala, Phe or naphthylalanine;
Xaa₇ is Thr or Ser;
20 Xaa₈ is Ala, Ser or Thr;
Xaa₉ is Asp or Glu;
Xaa₁₀ is Ala, Leu or pentylglycine;
Xaa₁₁ is Ala or Ser;
Xaa₁₂ is Ala or Lys;
25 Xaa₁₃ is Ala or Gln;
Xaa₁₄ is Ala, Leu or pentylglycine;
Xaa₁₅ is Ala or Glu;
Xaa₁₆ is Ala or Glu;
Xaa₁₇ is Ala or Glu;
30 Xaa₁₉ is Ala or Val;
Xaa₂₀ is Ala or Arg;
Xaa₂₁ is Ala or Leu;
Xaa₂₂ is Phe or naphthylalanine;

- Xaa₂₃ is Ile, Val or tert-butylglycine;
Xaa₂₄ is Ala, Glu or Asp;
Xaa₂₅ is Ala, Trp, or Phe;
Xaa₂₆ is Ala or Leu;
5 Xaa₂₇ is Ala or Lys;
Xaa₂₈ is Ala or Asn;
Z₁ is -OH,
-NH₂,
Gly-Z₂,
10 Gly Gly -Z₂,
Gly Gly Xaa₃₁-Z₂,
Gly Gly Xaa₃₁ Ser-Z₂,
Gly Gly Xaa₃₁ Ser Ser-Z₂,
Gly Gly Xaa₃₁ Ser Ser Gly-Z₂,
15 Gly Gly Xaa₃₁ Ser Ser Gly Ala-Z₂,
Gly Gly Xaa₃₁ Ser Ser Gly Ala Xaa₃₆-Z₂,
Gly Gly Xaa₃₁ Ser Ser Gly Ala Xaa₃₆ Xaa₃₇-Z₂ or Gly Gly
Xaa₃₁ Ser Ser Gly Ala Xaa₃₆ Xaa₃₇ Xaa₃₈-Z₂;
Xaa₃₁, Xaa₃₆, Xaa₃₇ and Xaa₃₈ are independently selected
20 from the group consisting of Pro, homoproline,
thiopropine and N-methylalanine; and
Z₂ is -OH or -NH₂;
provided that no more than three of Xaa₃, Xaa₅, Xaa₆, Xaa₈,
Xaa₁₀, Xaa₁₁, Xaa₁₂, Xaa₁₃, Xaa₁₄, Xaa₁₅, Xaa₁₆, Xaa₁₇, Xaa₁₉,
25 Xaa₂₀, Xaa₂₁, Xaa₂₄, Xaa₂₅, Xaa₂₆, Xaa₂₇ and Xaa₂₈ are Ala; and
pharmaceutically acceptable salts thereof.

FORMULA IV

- 30 Additionally, the present invention includes narrower
genera of peptide compounds described in PCT Application
Serial No. PCT/US98/24273, filed November 13, 1998, entitled
"Novel Exendin Agonist Compounds" as having particular amino
acid sequences, for example, compounds of the formula [IV]

[SEQ. ID. NO. 44]:

Xaa₁ Xaa₂ Xaa₃ Xaa₅ Xaa₅ Xaa₆ Xaa₇ Xaa₈ Xaa₉ Xaa₁₀ Xaa₁₁ Xaa₁₂
Xaa₁₃ Xaa₁₄ Xaa₁₅ Xaa₁₆ Xaa₁₇ Ala Xaa₁₈ Xaa₁₉ Xaa₂₀ Xaa₂₁ Xaa₂₂ Xaa₂₃
5 Xaa₂₄ Xaa₂₅ Xaa₂₆ Xaa₂₇ Xaa₂₈-Z₁; wherein

Xaa₁ is His or Ala;
Xaa₂ is Gly or Ala;
Xaa₃ is Ala, Asp or Glu;
10 Xaa₄ is Ala or Gly;
Xaa₅ is Ala or Thr;
Xaa₆ is Phe or naphthylalanine;
Xaa₇ is Thr or Ser;
Xaa₈ is Ala, Ser or Thr;
15 Xaa₉ is Ala, Asp or Glu;
Xaa₁₀ is Ala, Leu or pentylglycine;
Xaa₁₁ is Ala or Ser;
Xaa₁₂ is Ala or Lys;
Xaa₁₃ is Ala or Gln;
20 Xaa₁₄ is Ala, Leu, Met or pentylglycine;
Xaa₁₅ is Ala or Glu;
Xaa₁₆ is Ala or Glu;
Xaa₁₇ is Ala or Glu;
Xaa₁₉ is Ala or Val;
25 Xaa₂₀ is Ala or Arg;
Xaa₂₁ is Ala or Leu;
Xaa₂₂ is Phe or naphthylalanine;
Xaa₂₃ is Ile, Val or tert-butylglycine;
Xaa₂₄ is Ala, Glu or Asp;
30 Xaa₂₅ is Ala, Trp or Phe;
Xaa₂₆ is Ala or Leu;
Xaa₂₇ is Ala or Lys;
Xaa₂₈ is Ala or Asn;

- Z_1 is -OH,
 -NH₂,
 Gly-Z₂,
 Gly Gly-Z₂
 5 Gly Gly Xaa₃₁-Z₂,
 Gly Gly Xaa₃₁ Ser-Z₂,
 Gly Gly Xaa₃₁ Ser Ser-Z₂,
 Gly Gly Xaa₃₁ Ser Ser Gly-Z₂,
 Gly Gly Xaa₃₁ Ser Ser Gly Ala-Z₂,
 10 Gly Gly Xaa₃₁ Ser Ser Gly Ala Xaa₃₆-Z₂,
 Gly Gly Xaa₃₁ Ser Ser Gly Ala Xaa₃₆ Xaa₃₇-Z₂
 Gly Gly Xaa₃₁ Ser Ser Gly Ala Xaa₃₆ Xaa₃₇ Xaa₃₈-Z₂
 Gly Gly Xaa₃₁ Ser Ser Gly Ala Xaa₃₆ Xaa₃₇ Xaa₃₈
 Ser-Z₂;
 15 Xaa₃₁, Xaa₃₆, Xaa₃₇ and Xaa₃₈ are independently Pro,
 homoproline, thioproline, or
 N-methylalanine; and
 Z_2 is -OH or -NH₂;
 provided that no more than three of Xaa₃, Xaa₅, Xaa₆, Xaa₈,
 20 Xaa₁₀, Xaa₁₁, Xaa₁₂, Xaa₁₃, Xaa₁₄, Xaa₁₅, Xaa₁₆, Xaa₁₇, Xaa₁₉,
 Xaa₂₀, Xaa₂₁, Xaa₂₄, Xaa₂₅, Xaa₂₆, Xaa₂₇, and Xaa₂₈ are Ala; and
 provided that, if Xaa₁ is His, Arg or Tyr, then at least one
 of Xaa₃, Xaa₄ and Xaa₉ is Ala; and pharmaceutically
 acceptable salts thereof.
 25 Preferred compounds of formula (IV) include those
 wherein Xaa₁ is His, Ala, Norval or 4-imidazopropionyl.
 Preferably, Xaa₁ is His, or 4-imidazopropionyl or Ala, more
 preferably His or 4-imidazopropionyl.
 Preferred compounds of formula (IV) include those
 30 wherein Xaa₂ is Gly.
 Preferred compounds of formula (IV) include those
 wherein Xaa₄ is Ala.
 Preferred compounds of formula (IV) include those

wherein Xaa₉ is Ala.

Preferred compounds of formula (IV) include those wherein Xaa₁₄ is Leu, pentylglycine or Met.

Preferred compounds of formula (IV) include those
5 wherein Xaa₂₅ is Trp or Phe.

Preferred compounds of formula (IV) include those wherein Xaa₆ is Ala, Phe or naphthylalanine; Xaa₂₂ is Phe or naphthylalanine; and Xaa₂₃ is Ile or Val.

Preferred compounds of formula (IV) include those
10 wherein Z₁ is -NH₂.

Preferred compounds of formula (IV) include those wherein Xaa₃₁, Xaa₃₆, Xaa₃₇ and Xaa₃₈ are independently selected from the group consisting of Pro, homoproline, thioproline and N-alkylalanine.

Preferred compounds of formula (IV) include those
15 wherein Xaa₃₉ is Ser or Tyr, preferably Ser.

Preferred compounds of formula (IV) include those wherein Z₂ is -NH₂.

Preferred compounds of formula (IV) include those 42
20 wherein Z₁ is -NH₂.

Preferred compounds of formula (IV) include those wherein Xaa₂₁ is Lys-NH^e-R where R is Lys, Arg, C₁-C₁₀ straight chain or branched alkanoyl.

Preferred compounds of formula (IV) include those
25 wherein X₁ is Lys Asn, Lys-NH^e-R Asn, or Lys-NH^e-R Ala where R is Lys, Arg, C₁-C₁₀ straight chain or branched alkanoyl.
Preferred compounds of formula (IV) include those having an amino acid sequence described in PCT application Serial No. PCT/US98/24273, filed November 13, 1998, entitled "Novel
30 Exendin Agonist Compounds" as being selected from SEQ. ID. NOS. 95-110 therein.

FORMULA V

Also provided are compounds described in PCT application PCT/US98/24210, filed November 13, 1998, entitled "Novel Exendin Agonist Compounds", including
5 compounds of the formula (V) [SEQ. ID. NO. 45]:

5

10

Xaa₁ Xaa₂ Xaa₃ Gly Xaa₅ Xaa₆ Xaa₇ Xaa₈ Xaa₉ Xaa₁₀
Xaa₁₁ Xaa₁₂ Xaa₁₃ Xaa₁₄ Xaa₁₅ Xaa₁₆ Xaa₁₇ Ala Xaa₁₉ Xaa₂₀
Xaa₂₁ Xaa₂₂ Xaa₂₃ Xaa₂₄ Xaa₂₅ Xaa₂₆ X₁ -Z₁; wherein

10

Xaa₁ is His, Arg or Tyr or 4-imidazopropionyl;

Xaa₂ is Ser, Gly, Ala or Thr;

Xaa₃ is Asp or Glu;

Xaa₅ is Ala or Thr;

15 Xaa₆ is Ala, Phe, Tyr or naphthylalanine;

Xaa₇ is Thr or Ser;

Xaa₈ is Ala, Ser or Thr;

Xaa₉ is Asp or Glu;

Xaa₁₀ is Ala, Leu, Ile, Val, pentylglycine or Met;

20 Xaa₁₁ is Ala or Ser;

Xaa₁₂ is Ala or Lys;

Xaa₁₃ is Ala or Gln;

Xaa₁₄ is Ala, Leu, Ile, pentylglycine, Val or Met;

Xaa₁₅ is Ala or Glu;

25 Xaa₁₆ is Ala or Glu;

Xaa₁₇ is Ala or Glu;

Xaa₁₉ is Ala or Val;

Xaa₂₀ is Ala or Arg;

Xaa₂₁ is Ala, Leu or Lys-NH^e-R where R is Lys, Arg, C₁-C₁₀

30 straight chain or branched alkanoyl or cycloalkylalkanoyl;

Xaa₂₂ is Phe, Tyr or naphthylalanine;

Xaa₂₃ is Ile, Val, Leu, pentylglycine, tert-butylglycine
or Met;

Xaa₂₄ is Ala, Glu or Asp;

Xaa₂₅ is Ala, Trp, Phe, Tyr or naphthylalanine;

Xaa₂₆ is Ala or Leu;

X₁ is Lys Asn, Asn Lys, Lys-NH^e-R Asn, Asn Lys-NH^e-R, Lys-NH^e-

5 R Ala, Ala Lys-NH^e-R where R is Lys, Arg, C₁-C₁₀ straight chain or branched alkanoyl or cycloalkylalkanoyl

Z₁ is -OH,

-NH₂,

Gly-Z₂,

10 Gly Gly-Z₂,

Gly Gly Xaa₃₁-Z₂,

Gly Gly Xaa₃₁ Ser-Z₂,

Gly Gly Xaa₃₁ Ser Ser-Z₂,

Gly Gly Xaa₃₁ Ser Ser Gly-Z₂,

15 Gly Gly Xaa₃₁ Ser Ser Gly Ala-Z₂,

Gly Gly Xaa₃₁ Ser Ser Gly Ala Xaa₃₆-Z₂,

Gly Gly Xaa₃₁ Ser Ser Gly Ala Xaa₃₆ Xaa₃₇-Z₂ or

Gly Gly Xaa₃₁ Ser Ser Gly Ala Xaa₃₆ Xaa₃₇ Xaa₃₈-Z₂;

wherein

20 Xaa₃₁, Xaa₃₆, Xaa₃₇ and Xaa₃₈ are independently selected from the group consisting of Pro, homoproline, 3Hyp, 4Hyp, thioproline, N-alkylglycine, N-alkylpentylglycine and N-alkylalanine; and

25 Z₂ is -OH or -NH₂;

provided that no more than three of Xaa₃, Xaa₅, Xaa₆, Xaa₈, Xaa₁₀, Xaa₁₁, Xaa₁₂, Xaa₁₃, Xaa₁₄, Xaa₁₅, Xaa₁₆, Xaa₁₇, Xaa₁₉, Xaa₂₀, Xaa₂₁, Xaa₂₄, Xaa₂₅, and Xaa₂₆ are Ala. Also within the scope of the present invention are pharmaceutically

30 acceptable salts of the compound of formula (V) and pharmaceutical compositions including said compounds and salts thereof.

Preferred exendin agonist compounds of formula (V)

include those wherein Xaa₁ is His, Tyr or 4-imidazopropionyl. More preferably Xaa₁ is His.

Preferred are those compounds of formula (V) wherein Xaa₁ is 4-imidazopropionyl.

- 5 Preferred are those compounds of formula (V) wherein Xaa₂ is Gly.

Preferred compounds of formula (V) are those wherein Xaa₁₄ is Leu, pentylglycine or Met.

- 10 Preferred compounds of formula (V) are those wherein Xaa₂₅ is Trp or Phe.

- According to one aspect, preferred are compounds of formula (V) wherein Xaa₆ is Phe or naphthylalanine; and Xaa₂₂ is Phe or naphthylalanine; and Xaa₂₃ is Ile or Val. More preferably, Z₁ is -NH₂. According to one aspect, especially preferred are such compounds of formula (V) wherein Xaa₃₁, Xaa₃₆, Xaa₃₇ and Xaa₃₈ are independently selected from the group consisting of Pro, homoproline, thioproline and N-alkylalanine. More preferably, Z₂ is -NH₂.
- 15

- Preferred compounds of formula (V) include those wherein X₁ is Lys Asn, Lys-NH^e-R Asn, or Lys-NH^e-R Ala where R is Lys, Arg, C₁-C₁₀ straight chain or branched alkanoyl. Preferred compounds of formula (V) include compounds described in PCT application Serial No. PCT/US98/24210, filed November 13, 1998, entitled "Novel Exendin Agonist Compounds" and identified therein as Compound Nos. 62-69.
- 20
- 25

Preferred such exendin agonist compounds include those wherein Xaa₁ is His, Ala or Norval. More preferably Xaa₁ is His or Ala. Most preferably Xaa₁ is His.

- Preferred are those compounds of formula (V) wherein Xaa₂ is Gly.
- 30

Preferred are those compounds of formula (V) wherein Xaa₃ is Ala.

Preferred are those compounds of formula (V) wherein Xaa₄ is Ala.

Preferred are those compounds of formula (V) wherein Xaa₉ is Ala.

- 5 Preferred are those compounds of formula (V) wherein Xaa₁₄ is Leu, pentylglycine or Met.

Preferred compounds of formula (V) are those wherein Xaa₂₅ is Trp or Phe.

- 10 Preferred compounds of formula (V) are those where Xaa₆ is Ala, Phe or naphthylalanine; Xaa₂₂ is Phe or naphthylalanine; and Xaa₂₃ is Ile or Val.

Preferred are compounds of formula (V) wherein Xaa₃₁, Xaa₃₆, Xaa₃₇ and Xaa₃₈ are independently selected from Pro, homoproline, thioproline and N-alkylalanine.

- 15 Preferably Z₁ is -NH₂.
Preferably Z₂ is -NH₂.

- According to one aspect, preferred are compounds of formula (V) wherein Xaa₁ is Ala, His or Tyr, more preferably Ala or His; Xaa₂ is Ala or Gly; Xaa₆ is Phe or naphthylalanine; Xaa₁₄ is Ala, Leu, pentylglycine or Met; Xaa₂₂ is Phe or naphthylalanine; Xaa₂₃ is Ile or Val; Xaa₃₁, Xaa₃₆, Xaa₃₇ and Xaa₃₈ are independently selected from Pro, homoproline, thioproline or N-alkylalanine; and Xaa₃₉ is Ser or Tyr, more preferably Ser. More preferably Z₁ is -NH₂.

- 25 According to an especially preferred aspect, especially preferred compounds include those of formula (V) wherein:
Xaa₁ is His or Ala; Xaa₂ is Gly or Ala; Xaa₃ is Ala, Asp or Glu; Xaa₄ is Ala or Gly; Xaa₅ is Ala or Thr; Xaa₆ is Phe or naphthylalanine; Xaa₇ is Thr or Ser; Xaa₈ is Ala, Ser or Thr;
30 Xaa₉ is Ala, Asp or Glu; Xaa₁₀ is Ala, Leu or pentylglycine; Xaa₁₁ is Ala or Ser; Xaa₁₂ is Ala or Lys; Xaa₁₃ is Ala or Gln; Xaa₁₄ is Ala, Leu, Met or pentylglycine; Xaa₁₅ is Ala or Glu; Xaa₁₆ is Ala or Glu; Xaa₁₇ is Ala or Glu; Xaa₁₉ is Ala or Val;

Xaa₂₀ is Ala or Arg; Xaa₂₁ is Ala or Leu; Xaa₂₂ is Phe or naphthylalanine; Xaa₂₃ is Ile, Val or tert-butylglycine; Xaa₂₄ is Ala, Glu or Asp; Xaa₂₅ is Ala, Trp or Phe; Xaa₂₆ is Ala or Leu; Xaa₂₇ is Ala or Lys; Xaa₂₈ is Ala or Asn; Z₁ is -OH, -NH₂, Gly-Z₂, Gly Gly-Z₂, Gly Gly Xaa₃₁-Z₂, Gly Gly Xaa₃₁ Ser-Z₂, Gly Gly Xaa₃₁ Ser Ser-Z₂, Gly Gly Xaa₃₁ Ser Ser Gly-Z₂, Gly Gly Xaa₃₁ Ser Ser Gly Ala-Z₂, Gly Gly Xaa₃₁ Ser Ser Gly Ala Xaa₃₆-Z₂, Gly Gly Xaa₃₁ Ser Ser Gly Ala Xaa₃₆ Xaa₃₇-Z₂, Gly Gly Xaa₃₁ Ser Ser Gly Ala Xaa₃₆ Xaa₃₇ Xaa₃₈-Z₂ or Gly Gly Xaa₃₁ Ser Ser Gly Ala Xaa₃₆ Xaa₃₇ Xaa₃₈ Xaa₃₉-Z₂; Xaa₃₁, Xaa₃₆, Xaa₃₇ and Xaa₃₈ being independently Pro homoproline, thioproline or N-methylalanine; and Z₂ being -OH or -NH₂; provided that no more than three of Xaa₃, Xaa₅, Xaa₆, Xaa₈, Xaa₁₀, Xaa₁₁, Xaa₁₂, Xaa₁₃, Xaa₁₄, Xaa₁₅, Xaa₁₆, Xaa₁₇, Xaa₁₉, Xaa₂₀, Xaa₂₁, Xaa₂₄, Xaa₂₅, Xaa₂₆, Xaa₂₇ and Xaa₂₈ are Ala; and provided also that, if Xaa₁ is His, Arg or Tyr, then at least one of Xaa₃, Xaa₄ and Xaa₉ is Ala. Especially preferred compounds of formula (V) include those described in PCT application Serial No. PCT/US98/24210, filed November 13, 1998, entitled "Novel Exendin Agonist Compounds" and having the amino acid sequences identified therein as SEQ. ID. NOS. 5-93.

According to an especially preferred aspect, provided are compounds of formula (V) where Xaa₁₄ is Ala, Leu, Ile, Val or pentylglycine, more preferably Leu or pentylglycine, and Xaa₂₅ is Ala, Phe, Tyr or naphthylalanine, more preferably Phe or naphthylalanine. These compounds will be less susceptible to oxidative degradation, both in vitro and in vivo, as well as during synthesis of the compound.

30 FORMULA VI

Also provided are peptide compounds described in PCT Application Serial No. PCT/US98/24273, filed November 13, 1998, entitled "Novel Exendin Agonist Compounds", including

compounds of the formula (VI) [SEQ. ID. NO. 46]:

5

10

Xaa₁ Xaa₂ Xaa₃ Xaa₄ Xaa₅ Xaa₆ Xaa₇ Xaa₈ Xaa₉ Xaa₁₀

Xaa₁₁ Xaa₁₂ Xaa₁₃ Xaa₁₄ Xaa₁₅ Xaa₁₆ Xaa₁₇ Ala Xaa₁₉ Xaa₂₀

5 Xaa₂₁ Xaa₂₂ Xaa₂₃ Xaa₂₄ Xaa₂₅ Xaa₂₆ X₁-Z₁; wherein

Xaa₁ is His, Arg, Tyr, Ala, Norval, Val, Norleu or 4-imidazopropionyl;

Xaa₂ is Ser, Gly, Ala or Thr;

Xaa₃ is Ala, Asp or Glu;

10 Xaa₄ is Ala, Norval, Val, Norleu or Gly;

Xaa₅ is Ala or Thr;

Xaa₆ is Phe, Tyr or naphthylalanine;

Xaa₇ is Thr or Ser;

Xaa₈ is Ala, Ser or Thr;

15 Xaa₉ is Ala, Norval, Val, Norleu, Asp or Glu;

Xaa₁₀ is Ala, Leu, Ile, Val, pentylglycine or Met;

Xaa₁₁ is Ala or Ser;

Xaa₁₂ is Ala or Lys;

Xaa₁₃ is Ala or Gln;

20 Xaa₁₄ is Ala, Leu, Ile, pentylglycine, Val or Met;

Xaa₁₅ is Ala or Glu;

Xaa₁₆ is Ala or Glu;

Xaa₁₇ is Ala or Glu;

Xaa₁₉ is Ala or Val;

25 Xaa₂₀ is Ala or Arg;

Xaa₂₁ is Ala, Leu or Lys-NH^c-R where R is Lys, Arg, C¹⁻¹⁰

straight chain or branched alkanoyl or cycloalyleyl-alkanoyl;

Xaa₂₂ is Phe, Tyr or naphthylalanine;

Xaa₂₃ is Ile, Val, Leu, pentylglycine, tert-butylglycine or

30 Met;

Xaa₂₄ is Ala, Glu or Asp;

Xaa₂₅ is Ala, Trp, Phe, Tyr or naphthylalanine;

Xaa₂₆ is Ala or Leu;

X₁ is Lys Asn, Asn Lys, Lys-NH^e-R Asn, Asn Lys-NH^e-R, Lys-NH^e-R Ala, Ala Lys-NH^e-R where R is Lys, Arg, C₁-C₁₀ straight chain or branched alkanoyl or cycloalkylalkanoyl

Z₁ is -OH,

5 -NH₂,

Gly-Z₂,

Gly Gly-Z₂,

Gly Gly Xaa₃₁-Z₂,

Gly Gly Xaa₃₁ Ser-Z₂,

10 Gly Gly Xaa₃₁ Ser Ser-Z₂,

Gly Gly Xaa₃₁ Ser Ser Gly-Z₂,

Gly Gly Xaa₃₁ Ser Ser Gly Ala-Z₂,

Gly Gly Xaa₃₁ Ser Ser Gly Ala Xaa₃₆-Z₂,

Gly Gly Xaa₃₁ Ser Ser Gly Ala Xaa₃₆ Xaa₃₇-Z₂,

15 Gly Gly Xaa₃₁ Ser Ser Gly Ala Xaa₃₆ Xaa₃₇ Xaa₃₈-Z₂ or

Gly Gly Xaa₃₁ Ser Ser Gly Ala Xaa₃₆ Xaa₃₇ Xaa₃₈ Xaa₃₉-Z₂;

wherein

Xaa₃₁, Xaa₃₆, Xaa₃₇ and Xaa₃₈ are independently selected from the group consisting of Pro,

20 homoproline, 3Hyp, 4Hyp, thioproline,

N-alkylglycine, N-alkylpentylglycine and

N-alkylalanine; and

Z₂ is -OH or -NH₂;

provided that no more than three of Xaa₃, Xaa₄, Xaa₅, Xaa₆,

25 Xaa₈, Xaa₉, Xaa₁₀, Xaa₁₁, Xaa₁₂, Xaa₁₃, Xaa₁₄, Xaa₁₅, Xaa₁₆,

Xaa₁₇, Xaa₁₉, Xaa₂₀, Xaa₂₁, Xaa₂₄, Xaa₂₅, Xaa₂₆, are Ala; and

provided also that, if Xaa₁ is His, Arg, Tyr, or 4-

imidazopropionyl then at least one of Xaa₃, Xaa₄ and Xaa₉ is Ala.

30 Preferred compounds of formula (VI) include those wherein Xaa₁ is His, Ala, Norval or 4-imidazopropionyl.

Preferably, Xaa₁ is His, or 4-imidazopropionyl or Ala, more preferably His or 4-imidazopropionyl.

Preferred compounds of formula (VI) include those wherein Xaa₂ is Gly.

Preferred compounds of formula (VI) include those wherein Xaa₄ is Ala.

5 Preferred compounds of formula (VI) include those wherein Xaa₉ is Ala.

Preferred compounds of formula (VI) include those wherein Xaa₁₄ is Leu, pentylglycine or Met.

10 Preferred compounds of formula (VI) include those wherein Xaa₂₅ is Trp or Phe.

Preferred compounds of formula (VI) include those wherein Xaa₆ is Ala, Phe or naphthylalanine; Xaa₂₂ is Phe or naphthylalanine; and Xaa₂₃ is Ile or Val.

15 Preferred compounds of formula (VI) include those wherein Z₁ is -NH₂.

Preferred compounds of formula (VI) include those wherein Xaa₃₁, Xaa₃₆, Xaa₃₇ and Xaa₃₈ are independently selected from the group consisting of Pro, homoproline, thioproline and N-alkylalanine.

20 Preferred compounds of formula (VI) include those wherein Xaa₃₉ is Ser or Tyr, preferably Ser.

Preferred compounds of formula (VI) include those wherein Z₂ is -NH₂.

25 Preferred compounds of formula (VI) include those wherein Z₁ is -NH₂.

Preferred compounds of formula (VI) include those wherein Xaa₂₁ is Lys-NH^e-R where R is Lys, Arg, C₁-C₁₀ straight chain or branched alkanoyl.

30 Preferred compounds of formula (VI) include those wherein X₁ is Lys Asn, Lys-NH^e-R Asn, or Lys-NH^e-R Ala where R is Lys, Arg, C₁-C₁₀ straight chain or branched alkanoyl.

Preferred compounds of formula (VI) include those described in PCT Application Serial No. PCT/US98/24273,

filed November 13, 1998, entitled "Novel Exendin Agonist Compounds" as having an amino acid sequence selected from those identified therein as SEQ. ID. NOS. 95-110.

5 FORMULA VII

Compounds particularly useful according to the present invention are exendin agonist compounds described in U.S. Patent Application Serial No. 09/003,869, filed January 7, 1998, entitled "Use of Exendins And Agonists Thereof For The
10 Reduction of Food Intake", including compounds of the formula (VII) [SEQ. ID. NO. 47]:

1	5	10
Xaa ₁	Xaa ₂	Xaa ₃
Gly	Thr	Xaa ₄
Xaa ₅	Xaa ₆	Xaa ₇
Xaa ₈		
15	20	
Ser	Lys	Gln
Xaa ₉	Glu	Glu
Glu	Ala	Val
Arg	Leu	
25	30	
Xaa ₁₀	Xaa ₁₁	Xaa ₁₂
Xaa ₁₃	Leu	Lys
Asn	Gly	Gly
Xaa ₁₄		
35		
Ser	Ser	Gly
Ala	Xaa ₁₅	Xaa ₁₆
Xaa ₁₇	Xaa ₁₈	-Z

20 wherein Xaa₁ is His, Arg or Tyr; Xaa₂ is Ser, Gly, Ala or Thr; Xaa₃ is Asp or Glu; Xaa₄ is Phe, Tyr or naphthalanine; Xaa₅ is Thr or Ser; Xaa₆ is Ser or Thr; Xaa₇ is Asp or Glu; Xaa₈ is Leu, Ile, Val, pentylglycine or Met; Xaa₉ is Leu,
25 Ile, pentylglycine, Val or Met; Xaa₁₀ is Phe, Tyr or naphthalanine; Xaa₁₁ is Ile, Val, Leu, pentylglycine, tert-butylglycine or Met; Xaa₁₂ is Glu or Asp; Xaa₁₃ is Trp, Phe, Tyr, or naphthylalanine; Xaa₁₄, Xaa₁₅, Xaa₁₆ and Xaa₁₇ are independently Pro, homoproline, 3Hyp, 4Hyp, thioproline, N-alkylglycine, N-alkylpentylglycine or N-alkylalanine; Xaa₁₈
30 is Ser, Thr or Tyr; and Z is -OH or -NH₂; with the proviso that the compound does not have the formula of either SEQ. ID. NOS. 1 or 2. Preferred N-alkyl groups for N-

alkylglycine, N-alkylpentylglycine and N-alkylalanine include lower alkyl groups preferably of 1 to about 6 carbon atoms, more preferably of 1 to 4 carbon atoms. Suitable compounds include those having amino acid sequences of SEQ.

- 5 ID. NOS. 10 to 40. Also useful in the present invention are pharmaceutically acceptable salts of the compounds of formula (VII).

Preferred exendin agonist compounds include those wherein Xaa₁ is His or Tyr. More preferably Xaa₁ is His.

- 10 Preferred are those compounds wherein Xaa₂ is Gly.

Preferred are those compounds wherein Xaa₉ is Leu, pentylglycine or Met.

Preferred compounds include those wherein Xaa₁₃ is Trp or Phe.

- 15 Also preferred are compounds where Xaa₄ is Phe or naphthalanine; Xaa₁₁ is Ile or Val and Xaa₁₄, Xaa₁₅, Xaa₁₆ and Xaa₁₇ are independently selected from Pro, homoproline, thioproline or N-alkylalanine. Preferably N-alkylalanine has a N-alkyl group of 1 to about 6 carbon atoms.

- 20 According to an especially preferred aspect, Xaa₁₅, Xaa₁₆ and Xaa₁₇ are the same amino acid residue.

Preferred are compounds wherein Xaa₁₈ is Ser or Tyr, more preferably Ser.

Preferably Z is -NH₂.

- 25 According to one aspect, preferred are compounds of formula (VII) wherein Xaa₁ is His or Tyr, more preferably His; Xaa₂ is Gly; Xaa₄ is Phe or naphthalanine; Xaa₉ is Leu, pentylglycine or Met; Xaa₁₀ is Phe or naphthalanine; Xaa₁₁ is Ile or Val; Xaa₁₄, Xaa₁₅, Xaa₁₆ and Xaa₁₇ are independently
30 selected from Pro, homoproline, thioproline or N-alkylalanine; and Xaa₁₈ is Ser or Tyr, more preferably Ser. More preferably Z is -NH₂.

According to an especially preferred aspect, especially preferred compounds include those of formula (VII) wherein: Xaa₁ is His or Arg; Xaa₂ is Gly; Xaa₃ is Asp or Glu; Xaa₄ is Phe or naphthylalanine; Xaa₅ is Thr or Ser; Xaa₆ is Ser or Thr; Xaa₇ is Asp or Glu; Xaa₈ is Leu or pentylglycine; Xaa₉ is Leu or pentylglycine; Xaa₁₀ is Phe or naphthylalanine; Xaa₁₁ is Ile, Val or t-butyltylglycine; Xaa₁₂ is Glu or Asp; Xaa₁₃ is Trp or Phe; Xaa₁₄, Xaa₁₅, Xaa₁₆, and Xaa₁₇ are independently Pro, homoproline, thioproline, or N-methylalanine; Xaa₁₈ is Ser or Tyr; and Z is -OH or -NH₂; with the proviso that the compound does not have the formula of either SEQ. ID. NOS. 1 or 2. More preferably Z is -NH₂. Especially preferred compounds include those having the amino acid sequence of SEQ. ID. NOS. 10, 11, 22, 23, 24, 27, 29, 36, 37 and 40.

According to an especially preferred aspect, provided are compounds where Xaa₉ is Leu, Ile, Val or pentylglycine, more preferably Leu or pentylglycine, and Xaa₁₃ is Phe, Tyr or naphthylalanine, more preferably Phe or naphthylalanine. These compounds are believed to exhibit advantageous duration of action and to be less subject to oxidative degradation, both in vitro and in vivo, as well as during synthesis of the compound.

25 FORMULA VIII

Also provided are compounds described in PCT Application Serial No. PCT/US98/16387, filed August 6, 1998, entitled "Novel Exendin Agonist Compounds", including compounds of the formula (VIII) [SEQ. ID. NO. 48]:

30	1	5	10
	Xaa ₁ Xaa ₂ Xaa ₃ Gly Thr Xaa ₄ Xaa ₅ Xaa ₆ Xaa ₇ Xaa ₈		
		15	20
	Ser Lys Gln Xaa ₉ Glu Glu Glu Ala Val Arg Leu		

25

30

Xaa₁₀ Xaa₁₁ Xaa₁₂ Xaa₁₃ Leu X₁ Gly Gly Xaa₁₄

35

Ser Ser Gly Ala Xaa₁₅ Xaa₁₆ Xaa₁₇ Xaa₁₈-Z

- 5 wherein Xaa₁ is His, Arg, Tyr or 4-imidazopropionyl; Xaa₂ is Ser, Gly, Ala or Thr; Xaa₃ is Asp or Glu; Xaa₄ is Phe, Tyr or naphthylalanine; Xaa₅ is Thr or Ser; Xaa₆ is Ser or Thr; Xaa₇ is Asp or Glu; Xaa₈ is Leu, Ile, Val, pentylglycine or Met; Xaa₉ is Leu, Ile, pentylglycine, Val or Met; Xaa₁₀ is Phe,
- 10 Tyr or naphthylalanine; Xaa₁₁ is Ile, Val, Leu, pentylglycine, tert-butylglycine or Met; Xaa₁₂ is Glu or Asp; Xaa₁₃ is Trp, Phe, Tyr, or naphthylalanine; X₁ is Lys Asn, Asn Lys, Lys-NH^e-R Asn, Asn Lys-NH^e-R where R is Lys, Arg, C₁-C₁₀ straight chain or branched alkanoyl or
- 15 cycloalkylalkanoyl; Xaa₁₄, Xaa₁₅, Xaa₁₆ and Xaa₁₇ are independently Pro, homoproline, 3Hyp, 4Hyp, thioproline, N-alkylglycine, N-alkylpentylglycine or N-alkylalanine; Xaa₁₈ is Ser, Thr or Tyr; and Z is -OH or -NH₂; with the proviso that the compound does not have the formula of either SEQ.
- 20 ID. NOS. 1 or 2. Suitable compounds of formula (VIII) include compounds described in PCT Application Serial No. PCT/US98/16387, filed August 6, 1998, entitled "Novel Exendin Agonist Compounds" having the amino acid sequences of SEQ. ID. NOS. 37-40 therein.

- 25 Preferred exendin agonist compounds of formula (VIII) include those wherein Xaa₁ is His, Tyr or 4-imidazopropionyl. More preferably, Xaa₁ is His or 4-imidazopropionyl.

Preferred are those compounds of formula (VIII) wherein Xaa₂ is Gly.

- 30 Preferred are those compounds of formula (VIII) wherein Xaa₉ is Leu, pentylglycine or Met.

Preferred are those compounds of formula (VIII) wherein Xaa₁₃ is Trp or Phe.

Preferred are those compounds of formula (VIII) wherein X_1 is Lys Asn, or Lys-NH^e-R Asn, where R is Lys, Arg, C₁-C₁₀ straight chain or branched alkanoyl.

Also preferred are compounds of formula (VIII) wherein
5 Xaa₄ is Phe or naphthylalanine; Xaa₁₀ is Phe or naphthylalanine; Xaa₁₁ is Ile or Val and Xaa₁₄, Xaa₁₅, Xaa₁₆ and Xaa₁₇ are independently selected from Pro, homoproline, thioproline or N-alkylalanine. According to an especially preferred aspect, Xaa₁₈ is Ser or Tyr. Preferred are those
10 such compounds wherein Xaa₁₈ is Ser. Preferably, Z is -NH₂.

According to one preferred aspect, preferred are compounds of formula (VIII) wherein Xaa₄ is Phe or naphthylalanine; Xaa₁₀ is Phe or naphthylalanine; Xaa₁₁ is Ile or Val, X_1 is Lys Asn, or Lys-NH^e-R Asn, where R is Lys, Arg, C₁-C₁₀ straight chain or branched alkanoyl and Xaa₁₄, Xaa₁₅,
15 Xaa₁₆ and Xaa₁₇ are independently selected from Pro, homoproline, thioproline or N-alkylalanine.

Preparation of Modified Exendins And Exendin Agonists

20 The modified exendins and exendin agonists of the present invention may be made by linking one or more polyethylene glycol polymers to an exendin or exendin agonist. The synthesis of exendins and exendin agonists is thus described first, followed by methodology for linking
25 the polyethylene glycol polymer(s) to the exendin or exendin agonist.

Preparation of Exendins And Exendin Agonists

30 Exendins and exendin agonist compounds such as exendin analogs and exendin derivatives, described herein may be prepared through peptide purification as described in, for example, Eng, et al., J. Biol. Chem. 265:20259-62, 1990; and Eng, et al., J. Biol. Chem. 267:7402-05, 1992, hereby

incorporated by reference herein. Alternatively, exendins and exendin agonist peptides may be prepared by methods known to those skilled in the art, for example, as described in Raufman, et al. (J. Biol. Chem. 267:21432-37, 1992), hereby incorporated by reference herein, using standard solid-phase peptide synthesis techniques and preferably an automated or semiautomated peptide synthesizer. The compounds that constitute active ingredients of the formulations and dosages of the present invention may be prepared using standard solid-phase peptide synthesis techniques and preferably an automated or semiautomated peptide synthesizer. Typically, using such techniques, an α -N-carbamoyl protected amino acid and an amino acid attached to the growing peptide chain on a resin are coupled at room temperature in an inert solvent such as dimethylformamide, N-methylpyrrolidinone or methylene chloride in the presence of coupling agents such as dicyclohexylcarbodiimide and 1-hydroxybenzotriazole in the presence of a base such as diisopropylethylamine. The α -N-carbamoyl protecting group is removed from the resulting peptide-resin using a reagent such as trifluoroacetic acid or piperidine, and the coupling reaction repeated with the next desired N-protected amino acid to be added to the peptide chain. Suitable N-protecting groups are well known in the art, with t-butyloxycarbonyl (tBoc) and fluorenylmethoxycarbonyl (Fmoc) being preferred herein.

The solvents, amino acid derivatives and 4-methylbenzhydryl-amine resin used in the peptide synthesizer may be purchased from Applied Biosystems Inc. (Foster City, CA). The following side-chain protected amino acids may be purchased from Applied Biosystems, Inc.: BSD-112344.1-Arg(Pmc), Boc-Thr(Bzl), Fmoc-Thr(t-Bu), Boc-Ser(Bzl), Fmoc-Ser(t-Bu), Boc-Tyr(BrZ), Fmoc-Tyr(t-Bu), Boc-Lys(Cl-Z),

Fmoc-Lys(Boc), Boc-Glu(Bzl), Fmoc-Glu(t-Bu), Fmoc-His(Trt), Fmoc-Asn(Trt), and Fmoc-Gln(Trt). Boc-His(BOM) may be purchased from Applied Biosystems, Inc. or Bachem Inc. (Torrance, CA). Anisole, dimethylsulfide, phenol, ethanedithiol, and thioanisole may be obtained from Aldrich Chemical Company (Milwaukee, WI). Air Products and Chemicals (Allentown, PA) supplies HF. Ethyl ether, acetic acid and methanol may be purchased from Fisher Scientific (Pittsburgh, PA).

10 Solid phase peptide synthesis may be carried out with an automatic peptide synthesizer (Model 430A, Applied Biosystems Inc., Foster City, CA) using the NMP/HOBt (Option 1) system and tBoc or Fmoc chemistry (see, Applied Biosystems User's Manual for the ABI 430A Peptide Synthesizer, Version 1.3B July 1, 1988, section 6, pp. 49-70, Applied Biosystems, Inc., Foster City, CA) with capping. Boc-peptide-resins may be cleaved with HF (-50°C to 0°C, 1 hour). The peptide may be extracted from the resin with alternating water and acetic acid, and the filtrates
15 lyophilized. The Fmoc-peptide resins may be cleaved according to standard methods (Introduction to Cleavage Techniques, Applied Biosystems, Inc., 1990, pp. 6-12). Peptides may also be assembled using an Advanced Chem Tech Synthesizer (Model MPS 350, Louisville, Kentucky).

25 Peptides may be purified by RP-HPLC (preparative and analytical) using a Waters Delta Prep 3000 system. A C4, C8 or C18 preparative column (10 μ , 2.2 x 25 cm; Vydac, Hesperia, CA) may be used to isolate peptides, and purity may be determined using a C4, C8 or C18 analytical column (5
30 μ , 0.46 x 25 cm; Vydac). Solvents (A=0.1% TFA/water and B=0.1% TFA/CH₃CN) may be delivered to the analytical column at a flowrate of 1.0 ml/min and to the preparative column at 15 ml/min. Amino acid analyses may be performed on the

Waters Pico Tag system and processed using the Maxima program. Peptides may be hydrolyzed by vapor-phase acid hydrolysis (115°C, 20-24 h). Hydrolysates may be derivatized and analyzed by standard methods (Cohen, et al., The Pico Tag Method: A Manual of Advanced Techniques for Amino Acid Analysis, pp. 11-52, Millipore Corporation, Milford, MA (1989)). Fast atom bombardment analysis may be carried out by M-Scan, Incorporated (West Chester, PA). Mass calibration may be performed using cesium iodide or cesium iodide/glycerol. Plasma desorption ionization analysis using time of flight detection may be carried out on an Applied Biosystems Bio-Ion 20 mass spectrometer. Electrospray mass spectroscopy may be carried and on a VG-Trio machine.

Peptide active ingredient compounds useful in the formulations and dosages of the invention may also be prepared using recombinant DNA techniques, using methods now known in the art. See, e.g., Sambrook et al., Molecular Cloning: A Laboratory Manual, 2d Ed., Cold Spring Harbor (1989). Alternatively, such compounds may be prepared by homogeneous phase peptide synthesis methods. Non-peptide compounds useful in the present invention may be prepared by art-known methods. For example, phosphate-containing amino acids and peptides containing such amino acids, may be prepared using methods known in the art. See, e.g., Bartlett and Landen, Biorg. Chem. 14:356-377 (1986).

Conjugation of Polyethylene Glycol Polymers

There are several strategies for coupling PEG to peptides/proteins. See, Int. J. Hematology 68:1 (1998); Bioconjugate Chem. 6:150 (1995); and Crit. Rev. Therap. Drug Carrier Sys. 9:249 (1992) all of which are incorporated herein by reference in their entirety. Those skilled in the

art, therefore, will be able to utilize such well-known techniques for linking one or more polyethylene glycol polymers to the exendins and exendin agonists described herein. Suitable polyethylene glycol polymers typically are commercially available or may be made by techniques well known to those skilled in the art. The polyethylene glycol polymers preferably have molecular weights between 500 and 20,000 and may be branched or straight chain polymers.

The attachment of a PEG on an intact peptide or protein can be accomplished by coupling to amino, carboxyl or thiol groups. These groups will typically be the N and C termini and on the side chains of such naturally occurring amino acids as lysine, aspartic acid, glutamic acid and cysteine. Since exendin 4 and other exendins and exendin agonists can be prepared by solid phase peptide chemistry techniques, a variety of moieties containing diamino and dicarboxylic groups with orthogonal protecting groups can be introduced for conjugation to PEG.

The present invention also provides for conjugation of an exendin or exendin agonist to one or more polymers other than polyethylene glycol which can regulate kidney clearance in a manner similar to polyethylene glycol. Examples of such polymers include albumin and gelatin. See, Gombotz and Pettit, *Bioconjugate Chem.*, 6:332-351, 1995, which is incorporated herein by reference in its entirety.

Utility

The formulations and dosages described herein are useful in view of their pharmacological properties. In particular, the compounds described herein possess activity as agents to reduce glucagon levels and suppress glucagon secretion, as evidenced by the ability to lower glucagon levels in animals and humans. They can be used to treat

conditions or diseases that can be alleviated by reducing glucagon levels and suppressing glucagon secretion.

The compounds referenced above may form salts with various inorganic and organic acids and bases. Such salts include salts prepared with organic and inorganic acids, for example, HCl, HBr, H₂SO₄, H₃PO₄, trifluoroacetic acid, acetic acid, formic acid, methanesulfonic acid, toluenesulfonic acid, maleic acid, fumaric acid and camphorsulfonic acid. Salts prepared with bases include ammonium salts, alkali metal salts, e.g., sodium and potassium salts, and alkali earth salts, e.g., calcium and magnesium salts. Acetate, hydrochloride, and trifluoroacetate salts are preferred. The salts may be formed by conventional means, as by reacting the free acid or base forms of the product with one or more equivalents of the appropriate base or acid in a solvent or medium in which the salt is insoluble, or in a solvent such as water which is then removed in vacuo or by freeze-drying or by exchanging the ions of an existing salt for another ion on a suitable ion exchange resin.

Formulation and Administration

Modified exendin and exendin agonist formulations and dosages of the invention are useful in view of their exendin-like effects, and may conveniently be provided in the form of formulations suitable for parenteral (including intravenous, intramuscular and subcutaneous) administration. Also described herein are formulations and dosages useful in alternative delivery routes, including oral, nasal, buccal, sublingual and pulmonary.

The feasibility of alternate routes of delivery for exendin-4 has been explored by measuring exendin-4 in the circulation in conjunction with observation of a biologic response, such as plasma glucose lowering in diabetic

animals, after administration. Passage of exendin-4 has been investigated across several surfaces, the respiratory tract (nasal, tracheal and pulmonary routes) and the gut (sublingual, gavage and intraduodenal routes). Biologic
5 effect and appearance of exendin-4 in blood have been observed with each route of administration via the respiratory tract, and with sublingual and gavaged peptide via the gastrointestinal tract. Intra-tracheal
10 administration, nasal administration, administration via the gut, and sublingual administration have all been described.

In some cases, it will be convenient to provide a modified exendin or exendin agonist and another anti-glucagon agent, such as an amylin or an amylin agonist, in a single composition or solution for administration together.
15 In other cases, it may be more advantageous to administer another anti-glucagon agent separately from the exendin, exendin agonist, or modified exendin or exendin agonist. In yet other cases, it may be beneficial to provide an exendin, exendin agonist, or modified exendin or exendin agonist
20 either co-formulated or separately with other glucagon lowering agents such as amylin. A suitable administration format may best be determined by a medical practitioner for each patient individually. Suitable pharmaceutically acceptable carriers and their formulation are described in
25 standard formulation treatises, e.g., Remington's Pharmaceutical Sciences by E.W. Martin. See also Wang, Y.J. and Hanson, M.A. "Parenteral Formulations of Proteins and Peptides: Stability and Stabilizers," Journal of Parenteral Science and Technology, Technical Report No. 10, Supp. 42:2S
30 (1988).

Compounds useful in the invention can be provided as parenteral compositions for injection or infusion. They can, for example, be suspended in an inert oil, suitably a

vegetable oil such as sesame, peanut, olive oil, or other acceptable carrier. Preferably, they are suspended in an aqueous carrier, for example, in an isotonic buffer solution at a pH of about 5.6 to 7.4. These compositions may be

5 sterilized by conventional sterilization techniques, or may be sterile filtered. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as pH buffering agents. Useful buffers include for example,

10 sodium acetate/acetic acid buffers. A form of repository or "depot" slow release preparation may be used so that therapeutically effective amounts of the preparation are delivered into the bloodstream over many hours or days following transdermal injection or delivery.

15 The desired isotonicity may be accomplished using sodium chloride or other pharmaceutically acceptable agents such as dextrose, boric acid, sodium tartrate, propylene glycol, polyols (such as mannitol and sorbitol), or other inorganic or organic solutes. Sodium chloride is preferred

20 particularly for buffers containing sodium ions.

The claimed compounds can also be formulated as pharmaceutically acceptable salts (e.g., acid addition salts) and/or complexes thereof. Pharmaceutically acceptable salts are non-toxic salts at the concentration at

25 which they are administered. The preparation of such salts can facilitate the pharmacological use by altering the physical-chemical characteristics of the composition without preventing the composition from exerting its physiological effect. Examples of useful alterations in physical

30 properties include lowering the melting point to facilitate transmucosal administration and increasing the solubility to facilitate the administration of higher concentrations of the drug.

Pharmaceutically acceptable salts include acid addition salts such as those containing sulfate, hydrochloride, phosphate, sulfamate, acetate, citrate, lactate, tartrate, methanesulfonate, ethanesulfonate, benzenesulfonate, p-toluenesulfonate, cyclohexylsulfamate and quinate.

Pharmaceutically acceptable salts can be obtained from acids such as hydrochloric acid, sulfuric acid, phosphoric acid, sulfamic acid, acetic acid, citric acid, lactic acid, tartaric acid, malonic acid, methanesulfonic acid, ethanesulfonic acid, benzenesulfonic acid, p-toluenesulfonic acid, cyclohexylsulfamic acid, and quinic acid. Such salts may be prepared by, for example, reacting the free acid or base forms of the product with one or more equivalents of the appropriate base or acid in a solvent or medium in which the salt is insoluble, or in a solvent such as water which is then removed in vacuo or by freeze-drying or by exchanging the ions of an existing salt for another ion on a suitable ion exchange resin.

Carriers or excipients can also be used to facilitate administration of the compound. Examples of carriers and excipients include calcium carbonate, calcium phosphate, various sugars such as lactose, glucose, or sucrose, or types of starch, cellulose derivatives, gelatin, vegetable oils, polyethylene glycols and physiologically compatible solvents. The compositions or pharmaceutical composition can be administered by different routes including intravenously, intraperitoneal, subcutaneous, and intramuscular, orally, topically, or transmucosally.

If desired, solutions of the above compositions may be thickened with a thickening agent such as methylcellulose. They may be prepared in emulsified form, either water in oil or oil in water. Any of a wide variety of pharmaceutically acceptable emulsifying agents may be employed including, for

example, acacia powder, a non-ionic surfactant (such as a Tween), or an ionic surfactant (such as alkali polyether alcohol sulfates or sulfonates, e.g., a Triton).

Compositions useful in the invention are prepared by
5 mixing the ingredients following generally accepted procedures. For example, the selected components may be simply mixed in a blender or other standard device to produce a concentrated mixture which may then be adjusted to the final concentration and viscosity by the addition of
10 water or thickening agent and possibly a buffer to control pH or an additional solute to control tonicity.

For use by the physician, the compounds will be provided in dosage unit form containing an amount of an exendin, exendin agonist, or modified exendin or exendin
15 agonist, with or without another anti-glucagon agent. Therapeutically effective amounts of an exendin, exendin agonist, or modified exendin or exendin agonist for use in the control of glucagon and in conditions in which glucagon levels are beneficially lowered or regulated are those that
20 decrease post-prandial blood glucagon levels as desired. In diabetic or glucose intolerant individuals, plasma glucagon levels may be higher than in normal individuals. In such individuals, beneficial reduction or "smoothing" of post-prandial blood glucagon levels, may be obtained. As will be
25 recognized by those in the field, an effective amount of therapeutic agent will vary with many factors including the age and weight of the patient, the patient's physical condition, the glucagon level or level of inhibition of glucagon suppression to be obtained, and other factors.

Chs B53 ~~Such pharmaceutical compositions are useful in causing glucagon to be lowered in a subject and may be used as well in other disorders where lowered or suppressed glucagon is beneficially reduced.~~

The effective daily anti-glucagon dose of the compounds will typically be in the range of 0.01 or 0.03 to about 5 mg/day, preferably about 0.01 or 0.5 to 2 mg/day and more preferably about 0.01 or 0.1 to 1 mg/day, for a 70 kg patient, administered in a single or divided doses. The exact dose to be administered is determined by the attending clinician and is dependent upon where the particular compound lies within the above quoted range, as well as upon the age, weight and condition of the individual.

Administration should begin at the first sign of symptoms or shortly after diagnosis of, for example, diabetes mellitus as manifested by elevated glucagon. Administration may be by injection, preferably subcutaneous or intramuscular. Orally active compounds may be taken orally, however dosages should be increased 5-10 fold.

Chs B1 *bu* ~~Generally, in treating or preventing elevated, inappropriate, or undesired post-prandial blood glucagon levels, the compounds of this invention may be administered to patients in need of such treatment in a dosage ranges similar to those given above, however, the compounds are administered more frequently, for example, one, two, or three times a day. Particularly preferred are the exendin and exendin agonist formulations and dosages and routes of administration thereof described commonly owned U.S. Provisional Application 60/116,380, entitled "Novel Exendin Agonist Formulations And Methods Of Administration Thereof," filed January 14, 1999 (and the corresponding PCT application claiming priority from it that was filed on January 14, 2000, Serial No. [not yet assigned]), and U.S. Provisional Application 60/[not yet assigned], entitled "Use of Exendins and Agonists Thereof for Modulation of Triglyceride Levels and Treatment of Dyslipidemia," filed January 14, 1999, from which this application claims.~~

~~priority and the disclosures of which have been incorporated~~
by reference in their entirety as if fully set forth
~~herein.~~

The optimal formulation and mode of administration of
5 compounds of the present application to a patient depend on
factors known in the art such as the particular disease or
disorder, the desired effect, and the type of patient.
While the compounds will typically be used to treat human
patients, they may also be used to treat similar or
10 identical diseases in other vertebrates such as other
primates, farm animals such as swine, cattle and poultry,
and sports animals and pets such as horses, dogs and cats.

To assist in understanding the present invention the
following Examples are included which describe the results
15 of a series of experiments. The experiments relating to
this invention should not, of course, be construed as
specifically limiting the invention and such variations of
the invention, now known or later developed, which would be
within the purview of one skilled in the art are considered
20 to fall within the scope of the invention as described
herein and hereinafter claimed.

EXAMPLE 1 - PREPARATION OF EXENDIN-3

His Ser Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu
25 Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly
Pro Ser Ser Gly Ala Pro Pro Pro Ser-NH₂ [SEQ. ID. NO. 1]

The above amidated peptide was assembled on 4-(2'-4'-
dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using
30 Fmoc-protected amino acids (Applied Biosystems, Inc.). In
general, single-coupling cycles were used throughout the
synthesis and Fast Moc (HBTU activation) chemistry was
employed. Deprotection (Fmoc group removal) of the growing

peptide chain was achieved using piperidine. Final deprotection of the completed peptide resin was achieved using a mixture of triethylsilane (0.2 mL), ethanedithiol (0.2 mL), anisole (0.2 mL), water (0.2 mL) and

5 trifluoroacetic acid (15 mL) according to standard methods (Introduction to Cleavage Techniques, Applied Biosystems, Inc.) The peptide was precipitated in ether/water (50 mL) and centrifuged. The precipitate was reconstituted in glacial acetic acid and lyophilized. The lyophilized

10 peptide was dissolved in water). Crude purity was about 75%.

Used in purification steps and analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN).

Chs B2

~~the solution containing peptide was applied to a~~

15 preparative C-18 column and purified (10% to 40% Solvent B in Solvent A over 40 minutes). Purity of fractions was determined isocratically using a C-18 analytical column. Pure fractions were pooled furnishing the above-identified peptide. Analytical RP-HPLC (gradient 30% to 60% Solvent B

20 in Solvent A over 30 minutes) of the lyophilized peptide gave product peptide having an observed retention time of 19.2 minutes.

EXAMPLE 2 - PREPARATION OF EXENDIN-4

25 His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly Pro Ser Ser Gly Ala Pro Pro Pro Ser-NH₂ [SEQ. ID. NO. 2]

The above amidated peptide was assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide

30 norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Exendin-3 as describe in Example 1. Used in

analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 36% to 46% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide gave product peptide having an observed retention
5 time of 14.9 minutes. Electrospray Mass Spectrometry (M): calculated 4186.6; found 4186.0 to 4186.8 (four lots).

EXAMPLE 3: CLEARANCE BY THE KIDNEY

The kidney can play a major role in the elimination of
10 some molecules (drugs, peptides, proteins). For some molecules, this process begins when the kidney filters the blood at the glomerulus to produce the ultrafiltrate described below. The glomerular filter discriminates not only on the basis of molecular weight but also by acting as
15 a negatively charged selective barrier, promoting retention of anionic compounds. The free fraction of molecules in the plasma (not protein bound) with a molecular weight less than 5kD and an effective radii less than 15 Å are easily filtered. For larger molecular weight molecules they are
20 filtered on a more restrictive and limited basis, principally by molecular size, structure and net charge. The cutoff point for glomerular filtration lies between albumin (67kD) which is retained and hemoglobin (68kD) which is filtered. Albumin, with an effective radius of about 36
25 Å is filtered less than 1% at the glomerulus.

Once in the glomerulus a molecule travels to the proximal tubule where it is either reabsorbed or it passes on through the loop of Henle to the distal tubule where collecting ducts drain the filtrate into the bladder.
30 Filtered proteins and peptides are typically cleaved by brush border enzymes in the proximal tubule, from where they are efficiently retrieved by sodium/amino cotransporters (scavenging pumps). Otherwise, molecules which are polar,

ionized and of large molecular weight will not be reabsorbed. Throughout this process metabolizing enzymes in the renal cortex (proximal tubules) may also degrade the molecule into more polar molecules, thereby increasing the probability for excretion into the urine. Many peptide hormones (for example, amylin, calcitonins) are degraded by passage through the renal circulation, presumably by vascular ectoenzymes accessible to the plasma, independently of the process of glomerular filtration. In those examples, rates of peptide clearance from the plasma are similar to the rate of renal plasma flow, which is ~3-fold greater than the rate of glomerular filtration.

Studies performed to identify plasma circulating metabolites of exendin-4 yielded very little evidence of proteolytic degradation; following large intravenous doses in animals, HPLC analysis of plasma showed only the presence of intact exendin, and negligible appearance of "daughter" peaks indicative of the buildup of degradation products. This is in contrast to other peptides studied (for example amylin and GLP-1) where the disappearance of the "parent" HPLC peak was associated with the appearance of "daughter" HPLC peaks, subsequently identified as subpeptide degradants. The absence of plasma degradants of exendin indicates little or no proteolysis at any site, including the renal circulation. Any clearance by the kidney, then, is via non-proteolytic means, namely filtration or active excretion (as occurs with para-amino hippurate).

Initial measurements of exendin clearance in man (120-130 mL/min), monkeys (~9 mL/min) and rats (3.2-4.4 mL/min) matched reported glomerular filtration rates in those species. To test whether renal filtration could be the principal mode of exendin elimination, studies were performed in overnight fasted nephrectomized male rats

infused with exendin at a constant rate. Steady-state plasma levels of exendin-4 were greatly increased in nephrectomized rats compared to rats with their kidneys intact. This data indicated that the kidney was responsible for at least 80% of the clearance of exendin 4 (see Figures 5 and 6). Exendin clearance rates in intact rats were, again, similar to glomerular filtration rates expected in those rats (4.2 mL/min). Taken together these results indicate that very little metabolism occurs systemically and that most of the clearance of exendin 4 is through the kidney via filtration (but not by renal intravascular proteolysis). The low amounts of immunoreactive full-length exendin in the urine are consistent with it being cleaved by brush border enzymes in the proximal tubule after filtration.

EXAMPLE 4 - EXENDIN-4 DECREASES GLUCAGON SECRETION DURING
HYPERGLYCEMIC CLAMPS IN DIABETIC FATTY ZUCKER RATS

Absolute or relative hyperglucagonemia is often a feature of, for example, type 1 and type 2 diabetes mellitus, and the suppression of excessive glucagon secretion in these and other conditions described or referred to herein is a potential benefit of therapy using glucagonostatic agents. In this Example, the effect of exendin-4 on glucagon secretion in male anaesthetized Diabetic Fatty Zucker (ZDF) rats was examined. Using an hyperinsulinemic hyperglycemic clamp protocol, factors tending to influence glucagon secretion were held constant. Plasma glucose was clamped at ~34mM 60 min before beginning intravenous infusions of saline (n=7) or exendin-4 (0.21µg + 2.1µg/mL/h; n=7). Plasma glucagon concentration measured prior to these infusions were similar in both groups (306 ± 30pM versus 252 ± 32pM, respectively; n.s.).

Mean plasma glucagon concentration in exendin-4 infused rats was nearly half of that in saline-infused rats in the final 60 minutes of the clamp ($165 \pm 18\text{pM}$ versus $298 \pm 26\text{pM}$, respectively; $P < 0.002$). The hyperglycemic clamp protocol
5 also enabled measurement of insulin sensitivity. Glucose infusion rate during the clamp was increased by $111 \pm 7\%$ in exendin-4-treated versus control rats ($P < 0.001$). In other words, exendin-4 exhibited a glucagonostatic effect in ZDF rats during hyperglycemic clamp studies, an effect that will
10 be of therapeutic benefit in diabetic humans.

EXAMPLE 5 - METABOLIC EFFECTS OF EXENDIN-4 ON GLUCAGON
SECRETION IN PEOPLE WITH TYPE 2 DIABETES

In this Example, the safety, tolerability, and efficacy
15 of synthetic exendin-4 was evaluated in 8 male non-insulin using patients with type 2 diabetes who had discontinued other antidiabetic therapy for a minimum of 7 days. Each patient received subcutaneous (SC) injections of placebo (PBO) and 0.1, 0.2, and 0.3 $\mu\text{g/kg}$ exendin-4 48 hours apart
20 in a single-blind, dose-rising, placebo controlled crossover design. Five patients also received a 0.4 $\mu\text{g/kg}$ dose. Plasma glucose, insulin and glucagon concentrations were assessed fasting and in response to a 7 Kcal/kg Sustacal® challenge administered at the time of exendin-4/PBO
25 injection. Gastric emptying was evaluated by measuring serum acetaminophen concentrations following a 20 mg/kg oral dose of liquid acetaminophen administered with the Sustacal®. No safety issues were identified based upon reported adverse events, EKG and safety lab monitoring.
30 Doses of 0.3 and 0.4 $\mu\text{g/kg}$ elicited a dose-dependent increase in nausea; vomiting occurred at the highest dose.

Plasma glucose concentrations were reduced in all doses of exendin-4 compared to PBO although insulin concentrations

were not significantly different. The 8 hour mean \pm SE changes in plasma glucose AUC from baseline were $+391 \pm 187$, -263 ± 108 , -247 ± 64 , -336 ± 139 , and -328 ± 70 mg*hr/dL for the PBO, 0.1, 0.2, 0.3, and 0.4 μ g/kg doses respectively. The 3 hr changes in plasma glucagon were $+128.0 \pm 19.2$, -5.6 ± 10.5 , -29.4 ± 18.6 , -40.5 ± 24.5 , and $+6.9 \pm 38.6$ pg*hr/mL respectively. The gastric emptying rate was slowed in all doses and the mean total absorbed acetaminophen over 6 hours was reduced by 51%, 50%, 57% and 79% compared to PBO for 0.1, 0.2, 0.3, and 0.4 μ g/kg doses respectively. In summary, SC injection of exendin-4 to patients identified no safety issues, was tolerated at doses ≤ 0.3 μ g/kg, reduced plasma glucose and glucagon and slowed the rate of gastric emptying. These observations support the use of exendin for the treatment of conditions that would benefit from reduced glucagon levels and/or suppression of glucagon, including but not limited to type 1 and type 2 diabetes.

EXAMPLE 6: PEG MODIFIED EXENDIN 4

In the case of exendin 4, a 39 amino acid peptide with a molecular weight of 4187, modifications that increase its size and/or increase its anionic nature will decrease its ability to be filtered by the kidney. Because clearance of exendin 4 is largely by the kidney this will effectively increase its half life. Other properties of PEGylation (increased plasma half-life due to evasion of such renal and/or cellular clearance mechanisms that may exist; reduced immunogenicity and antigenicity; increased solubility; resistance to proteolysis; reduced toxicity (avoid dose spike); improved thermal and mechanical stability; improved permeability of the mucus or epithelial layer; and selective control over a specific biological function) are also of potential benefit for exendin 4 and exendin agonists.

In particular, because we have observed multiple pharmacologies (likely representing multiple receptor subtypes), different spectra of biological activities of exendin 4 may be selected by putting a PEG group at appropriate positions. Loss or alteration of bioactivity has been reported for PEGylated proteins which may be due to the presence of the PEG chains themselves, the particular site occupied by the PEG chain, or the coupling conditions having an adverse effect on the protein.

Primary considerations for PEG modification in terms of filtration at the kidney of exendin and exendin agonists are size and charge. Unmodified, exendin 4 has a molecular weight of approximately 4.2 kD and is anionic in nature with an overall net charge of approximately -2 at physiological pH. One, two or three PEG constituents may be covalently linked to exendin 4 or an analog of exendin 4, for example, with one PEG constituent being preferred. The size of the PEG can vary from a molecular weight of 500 to 20,000, preferably between 5,000 and 12,000.

discovery
BB
~~Many of the methods for covalent attachment of PEG take advantage of the epsilon-amino group on lysine. Exendin 4 has two lysines that can be modified by attachment of PEG. An alanine scan of AC3177 (Leu¹⁴, Phe²⁵₁₋₂₈ exendin-4), a shortened analog of exendin 4, revealed positions that are sensitive to substitution by alanine. The two lysines at positions 12 and 27 were moderately affected by this substitution suggesting that loss of the lysine specific R group side chain (methylene chain plus epsilon-amino group) is tolerated. With regard to the full-length peptide, exendin 4, the two lysine positions are appropriate for PEG attachment (see compounds 1 and 2). In addition, depending on the chemistry used to conjugate the PEG, the epsilon-~~

~~amino groups at these positions may be masked thereby~~
 increasing the anionic nature of the peptide.

(201) HGE~~FT~~TS~~DL~~SK (PEG) QMEEEEAVRLFIEWLKN~~GG~~PSSGAPPPS-NH₂

~~(202) HGE~~FT~~TS~~DL~~SKOMEEEEAVRLFIEWLKN~~GG~~(PEG)PSSGAPPPS-NH₂~~

~~Based on the results of the alanine scan, other likely~~
 positions that may be modified by insertion of a Lys-PEG or
 equivalent, for example, are:

(203) HK (PEG) EGTFTSDLSKQME~~EE~~AVRLFIEWLKN~~GG~~PSSGAPPPS-NH₂

(204) HGE~~G~~K (PEG) FTSDLSKQME~~EE~~AVRLFIEWLKN~~GG~~PSSGAPPPS-NH₂

10 (205) HGE~~G~~TFTK (PEG) DLSKQME~~EE~~AVRLFIEWLKN~~GG~~PSSGAPPPS-NH₂

(206) HGE~~G~~TFTSDK (PEG) SKQME~~EE~~AVRLFIEWLKN~~GG~~PSSGAPPPS-NH₂

(207) HGE~~G~~TFTSDLK (PEG) KQME~~EE~~AVRLFIEWLKN~~GG~~PSSGAPPPS-NH₂

(208) HGE~~G~~TFTSDLSK (PEG) MEEEEAVRLFIEWLKN~~GG~~PSSGAPPPS-NH₂

(209) * HGE~~G~~TFTSDLSKQMEK (PEG) EAVRLFIEWLKN~~GG~~PSSGAPPPS-NH₂

15 (210) * HGE~~G~~TFTSDLSKQME~~E~~EK (PEG) AVRLFIEWLKN~~GG~~PSSGAPPPS-NH₂

(211) HGE~~G~~FTSDLSKQME~~EE~~EAK (PEG) RLFIEWLKN~~GG~~PSSGAPPPS-NH₂

(212) HGE~~G~~TFTSDLSKQME~~EE~~AVRK (PEG) FIEWLKN~~GG~~PSSGAPPPS-NH₂

(213) * HGE~~G~~TFTSDLSKQME~~EE~~AVRLF~~I~~K (PEG) WLKN~~GG~~PSSGAPPPS-NH₂

(214) HGE~~G~~TFTSDLSKQME~~EE~~AVRLF~~I~~EK (PEG) LKN~~GG~~PSSGAPPPS-NH₂

20 ~~(215) HGE~~G~~TFTSDLSKQME~~EE~~AVRLF~~I~~EK (PEG) LKN~~GG~~PSSGAPPPS-NH₂~~

~~The three positions* above normally containing a~~
 glutamic acid that were indicated for modification with
 K(PEG) can also be modified by conjugation to the glutamic
 side chain carboxyl group, E(PEG).

~~Another analog in which the Lys-PEG can be added is at~~
 the supposed GlyGly turn.

~~(216) HGE~~G~~FTSDLSKQME~~EE~~AVRLFIEWLKN~~GG~~(PEG)PSSGAPPPS-NH₂~~

~~(217) HGE~~G~~FTSDLSKQME~~EE~~AVRLFIEWLKN~~GG~~(PEG)PSSGAPPPS-NH₂~~

Positions 29-39 of exendin-4 may not be critical for the
 30 glucose lowering activity as evidenced by AC3177 having

nearly equipotent activity to exendin 4, and any of them, alone or in combination, can be substituted for K(PEG) or an equivalent.

5 One skilled in the art would readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The molecular complexes and the methods, procedures, treatments, molecules, specific
10 compounds described herein are presently representative of preferred embodiments are exemplary and are not intended as limitations on the scope of the invention. Changes therein and other uses will occur to those skilled in the art which are encompassed within the spirit of the invention are
15 defined by the scope of the claims.

It will be readily apparent to one skilled in the art that varying substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention.

20 All patents and publications mentioned in the specification are indicative of the levels of those skilled in the art to which the invention pertains. All patents and publications are herein incorporated by reference to the same extent as if each individual publication was
25 specifically and individually indicated to be incorporated by reference.

The invention illustratively described herein suitably may be practiced in the absence of any element or elements, limitation or limitations, which is not specifically
30 disclosed herein. Thus, for example, in each instance herein any of the terms "comprising", "consisting essentially of" and "consisting of" may be replaced with either of the other two terms. The terms and expressions

which have been employed are used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but
5 it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the concepts herein
10 disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims.

In addition, where features or aspects of the invention
15 are described in terms of Markush groups, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group. For example, if X is described as selected from the group consisting of bromine,
20 chlorine, and iodine, claims for X being bromine and claims for X being bromine and chlorine are fully described.

The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure
25 also form part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

30 Other embodiments are within the following claims.